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Anticonvulsant activity of raw and classically processed *Vacha* (*Acorus calamus* Linn.) rhizomes

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Abstract

The rhizome of *Vacha* (*Acorus calamus*) has been used in Ayurvedic medicine for the treatment of various ailments, such as epilepsy, headache, eye disorders, insomnia, loss of memory, etc. Previous studies demonstrated that *Vacha* rhizome is having significant anticonvulsant activity against various induced seizures models in experimental animals. Ayurvedic pharmacopoeia of India has advocated *Shodhana* (purificatory procedures) to be done prior to its use. In the present study a comparative anticonvulsant activity of raw and *Shodhita* (classically processed) *Vacha* rhizomes were screened against Maximal Electro Shock (MES) seizure model to assess the effect of classical purificatory procedure on pharmacological action of *Vacha*. Phenytoin was used as standard antiepileptic drug for comparison. Pretreatment with both raw and classically processed *Vacha* samples exhibited significant anticonvulsant activity by decreasing the duration of tonic extensor phase. Further classically processed *Vacha* statistically decreased the duration of convulsion and stupor phases of MES-induced seizures. The results obtained from the present study clearly confirmed the anticonvulsant activity of raw *Vacha* and subjecting to classical *Shodhana* procedure did not alter the efficacy of *Vacha* rhizomes instead it enhanced the activity profile of the *Vacha*.

Key words: *Acorus calamus*, anticonvulsant, epilepsy, phenytoin, *shodhana*, *vacha*

Introduction

Acorus calamus Linn. (Family: Acoraceae) is a semi-evergreen perennial medicinal plant with scented rhizomes, arching tapered reed-like leaves and minute yellow-green flowers. It is known as *Vacha* in Ayurveda and the rhizome of this plant has been used since ancient times for its beneficial role as brain tonic (*Medhya*).[1] It has also been reported to possess tranquilizing,[2-5] antimicrobial,[6] antidiarrheal,[7] antidyslipidemic,[8] neuroprotective,[9] antioxidant,[10] anticholinesterase,[11,12] spasmolytic,[13] antiulcer,[14] anthelmintic,[15] anti-inflammatory, and analgesic[16,17] activities. Most of these functions are attributed to the aromatic oil present in the rhizome.[18,19] Further the essential oil from *Acorus* has been reported to be having antiepileptic activity against seizures induced by various means.[20-24]

Ayurvedic classics have emphasized various methods of *Shodhana* (purificatory procedures) to overcome the undesired effects from various poisonous and nonpoisonous drugs.[25,26] Even though *Vacha* does not come under poisonous drug category, some Ayurvedic texts and Ayurvedic pharmacopoeia of India have recommended *Shodhana* for *Vacha* rhizome.[27,28] The reason behind this *Shodhana* procedure though clearly not mentioned by any of the texts, it may be presumed to reduce the *Tiksnata* and emetic actions of rhizome. Till date not a single work has been reported on activity profile of classically processed *Vacha*, hence in this study *Vacha* rhizomes were subjected to classical purificatory procedure and a comparative anticonvulsant activity evaluation of raw and classically processed rhizomes were undertaken as a preliminary study to evaluate the role of classical purificatory procedure in modifying the therapeutic efficacy.

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Materials and Methods

Plant materials

Rhizomes of *A. calamus* were collected from its natural habitat in the forest regions of Yelagiri hills, Tamilnadu in the month of November in fully matured condition.[29] The plant material was identified and authenticated by the Pharmacognosist

of IPGT & RA, Gujarat Ayurved University, Jamnagar. The roots and old leaf scars were removed, washed thoroughly in water to remove the soil adhered to it, and then dried in partial shade. The rhizomes were cut uniformly into smaller pieces and divided into two parts. The first part was coded as sample raw *Vacha* (RV) and the second part was utilized for *Shodhana*. The *Shodhana* procedure involved boiling of *Vacha* samples successively by *Gomutra*, *Mundi kwatha* (decoction prepared from whole plant of *Sphaeranthus indicus* Linn.), *Panchapallava kwatha* (decoction prepared from a group of five leaves), and *Gandhodaka* (decoction prepared from a group of aromatic herbs) as described in Ayurvedic text.[27] After *Shodhana* procedure the rhizomes were shade dried for 12 days and marked as sample *Shodhita Vacha* (SV). Then both RV and SV were powdered (Mesh 80) and utilized for screening of anticonvulsant activity.

Animals

Charles–Foster strain albino rats weighing 200 ± 10 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 polypropylene with stainless steel top grill. The dry wheat (posthulled) waste was used as bedding material and was changed every morning. 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 Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) guidelines. Animals were provided with balanced food (Amrut brand rat pellet feed supplied by Pranav Agro Mills Pvt. Limited) and water ad libitum. 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). The animals were fasted overnight before the experiment.

Dose selection and schedule

The dose of *Vacha* as per Ayurvedic Pharmacopoiea of India is 120 mg per day.[28] The dose for experimental animals was calculated by extrapolating the human dose to animals based on the body surface area ratio by referring to the standard table of Paget and Barnes.[30] On this basis the rat dose of *Vacha* samples (RV and SV) was found to be 10.80 mg/kg rat and is rounded to 11 mg/kg. The test drug was suspended in distilled water with suitable concentration depending on the body weight of animals and administered orally with the help of gastric catheter sleeved to syringe. Phenytoin sodium was selected as standard antiepileptic drug (RS) and administered in the dose of 25 mg/kg (i.p).[31]

Anticonvulsant activity against maximal electroshock seizures

The rats were pretested 24 h prior to administration of test drugs for sensitivity to electric shock and those failing to give hind limb tonic extension were rejected. Thus screened animals were divided in to four groups of six animals each. Group 1 served as control, received equivalent amount of the vehicle (distilled water). Group 2 and 3 received RV and SV samples of *Vacha*, respectively. Fourth group received phenytoin sodium as standard antiepileptic drug. Experiment was conducted at the same time each day and 60 min after vehicle/drug administration (Phenytoin was given 30 min before the application of electroshock). Seizures are induced to all the groups by using an Electro-convulsiometer and elicited by a 60 Hz alternating current of 150 mA intensity for 0.2s. A drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes prior to application to the rats. The duration of various phases of epilepsy were recorded.[32]

Statistical analysis

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

Results

Pretreatment with RV and SV exhibited significant anticonvulsant activity by decreasing the duration of tonic extensor phase [Table 1]. Further RV-treated group showed 31.76% protection, while SV showed 36.48% protection against MES induced seizures. Both the samples of *Vacha* shortened other phases of MES-induced seizures, such as flexion, convulsion, and stupor, however, only the observed decrease of clonus and stupor in SV-treated group was found to be statistically significant. The standard drug phenytoin had exhibited significant anticonvulsant effect by abolishing the tonic extension phase. Further it significantly shortened all other phases induced by MES.

Discussion

Maximal Electro-Shock seizure is one of the commonly used models for preliminary testing of anticonvulsant drugs that produces generalized tonic–clonic seizures. This test serves to identify compounds that prevent seizure spread, corresponding to generalized tonic–clonic seizures in humans.[33] It has often been stated that antiepileptic drugs that block MES-induced

Table 1: Effect on MES-induced seizures in rats

Groups	Flexion (s)	Extension (s)	Convulsion (s)	Stupor (s)	% protection
Control	5.16 ± 0.65	14.17 ± 0.87	52.00 ± 2.58	97.83 ± 6.40	---
RV	4.00 ± 0.73	09.67 ± 0.66*	49.67 ± 3.49	88.67 ± 4.05	31.76
SV	4.00 ± 0.58	09.00 ± 0.86*	42.33 ± 2.39*	81.00 ± 3.04*	36.48
Phenytoin	0.66 ± 0.42**	01.17 ± 0.54***	15.83 ± 2.86**	32.33 ± 2.80***	91.74
df=3	F=10.23	F=53.84	F=33.70	F=45.88	

Data: Mean ± SEM, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. RV- Raw *Vacha*, SV-*Shodhita Vacha*, s-seconds.

tonic extension act by blocking seizure spread, moreover MES-induced tonic extension can be prevented either by drugs that inhibit voltage dependent Na^+ channels or by drugs that block glutaminergic excitation mediated by the *N*-methyl-d-aspartate (NMDA) receptor.[34] Phenytoin is effective in therapy of generalized tonic-clonic and partial seizures have been found to show strong anticonvulsant action by increasing brain content of Gamma-Amino Butyric Acid (GABA) in MES test.[35,36] In the present study treatment with RV and SV significantly inhibited the MES-induced seizures. Because inhibition of the MES test predicts activity against generalized tonic–clonic and cortical focal seizures, activity against MES-induced seizures suggests that both RV and SV samples are useful in suppressing generalized tonic–clonic seizures by regulating GABA-mediated synaptic inhibition. Koo *et al.* and Liao *et al.* showed that pre-inhalation of the essential oil of *acorus* markedly delayed the appearance of pentylenetetrazole-induced convulsion by inhibiting the activity of gamma-amino butyric acid (GABA) transaminase.[23,24] The same mechanism may be involved in observed activity profile. Furthermore, pretreatment with SV significantly decreased the duration of convulsion and stupor phases of MES-induced seizures. This shows that SV sample is having a better anticonvulsant activity. This may be attributed to acquiring of some active principles from *shodhana dravya*, such as *gomutra* (cow urine) and *Mundi Kwatha* (decoction of *S. indicus* Linn.), which are reported to be having anticonvulsant activity.[37,38]

Conclusion

The results obtained from the present study clearly confirmed the anticonvulsant activity of *Vacha*. Subjecting to classical *Shodhana* procedure did not lessen the efficacy of *Vacha* rhizomes instead it enhanced the activity profile of the *Vacha*.

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हिन्दी सारांश

अशोधित एवं शास्त्रोक्त शोधित वचा कन्द के आक्षेपहर कर्म का अध्ययन

सविता डी. भट्ट, अशोक बी. के., आचार्य आर. एन., रविशंकर बी.

आयुर्वेदिक ग्रन्थों में वचा कन्द अनेक व्याधियों में उपयोगी माना गया है, जैसे कि अपस्मार, शिरःशूल, स्मृतिभ्रंश, नेत्ररोग, निद्रारोग आदि । विगत में हुए अध्ययन में वचा कन्द के सार्थक आक्षेपहर कर्म को दर्शाया गया है । आयुर्वेदिक फार्माकोपिया में वचा का शोधन आवश्यक बताया गया है । शास्त्रोक्त शोधन विधि का वचा के भेषजविज्ञानीय कर्म के प्रभाव का निरीक्षण करने हेतु अशोधित और शोधित वचा के कन्द का तुलनात्मक आक्षेपहर कर्म अध्ययन MES model पर किया गया । फेनिटाइन को मानक अपस्माररोधी औषधि के रूप में लिया गया । अशोधित और शोधित दोनों वचा कन्द के नमूनों को प्रायोगिक चूहों को परीक्षण पूर्व खिलाकर, पश्चात् आक्षेप उत्पन्न कर आक्षेपहर कर्म का आंकलन ५ दिन किया गया जो कि सांख्यिकीय दृष्टि से सार्थक पाया गया । यह tonic extensor phase के समय को कम करता है । शोधित वचा, आक्षेप के समय को सांख्यिकीय दृष्टि से पर्याप्त कम करता है और MES induced seizures के phase को stupor करता है । वर्तमान अध्ययन से यह निश्चित होता है कि अशोधित वचा के आक्षेपहर कर्म में, शास्त्रोक्त वचा शोधन प्रक्रिया से कोई परिवर्तन नहीं पाया गया, अपितु उसकी कार्मुकता में वृद्धि हुई ।