

# Ameliorative Effect of Methanolic Extract of *Allium cepa* on Anticonvulsant Activity of Chloroform Extract of *Acorus calamus* Linn. Rhizomes

Rahul Kaushik<sup>1</sup>, Jainendra Jain<sup>1</sup>, Rashmi Yadav<sup>2</sup>, Lubhan Singh<sup>1</sup>, Deepika Gupta<sup>1</sup>, Akanksha Gupta<sup>3</sup>

<sup>1</sup>Ram-Eesh Institute of Vocational and Technical Education, Plot No. 3, Knowledge Park- I, Kasna Road, Greater Noida, Gautam Budh Nagar- 201310, Uttar Pradesh, INDIA.

<sup>2</sup>Department of Pharmacy, Sharda University, Greater Noida, Uttar Pradesh, INDIA.

<sup>3</sup>Krishnarpit Institute of Pharmacy, Allahabad, Uttar Pradesh, INDIA.

## ABSTRACT

**Background:** *Acorus calamus* is used in Ayurvedic System of Medicine as Medhya rasayana and prescribed as an aphrodisiac and nerve tonic since ancient times. Methanolic extract of *Allium cepa* (AC) bulbs was reported to possess anti-oxidant activity. **Objective:** In this study we evaluated antiepileptic activity of chloroform extract containing  $\beta$ -Asarone (CBA) alone in varying doses and its combinations with varying doses of methanolic extract of *Allium cepa* bulbs (MAC) in Isoniazid induced seizure model in mice. **Method:** Isoniazid (INH, 150mg/kg) induced seizure model was used to study antiepileptic effects in mice. The mice were administered the test (CBA1, CBA2, CBA3, CBAC1, CBAC2 and CBAC3 and standard drug (Sodium valproate) 1hr before inducing seizures. The animals were observed for 2 hrs for onset of seizures and death. **Results:** Study has shown that CBA at a dose of 50, 100 and 150 mg/kg offered 50%, 83.33% and 100% protection against death respectively whereas CBAC, the combinations of CBA (50mg/kg) with 100, 200 and 300mg/kg dose of MAC shows 66.67%, 100% and 100% protection of animals against death respectively prolonging the mean time for onset of seizures and reducing the severity of sei-

zures as compared to CBA (50mg/kg) alone. **Conclusion:** The methanolic extract of *Allium cepa* ameliorated the anticonvulsant activity of chloroform extract containing  $\beta$ -Asarone and also reduced the severity of seizure episodes at all doses. The combined therapy of  $\beta$ -Asarone and *Allium cepa* can prove to be an alternative to synthetic antiepileptic drugs.

**Key words:** Anticonvulsant, *Acorus calamus*, Isoniazid (INH),  $\beta$ -Asarone, *Allium cepa*.

## Correspondence:

Rahul Kaushik,

Assistant Professor, Ram-Eesh Institute of Vocational and Technical Education, Plot No. 3, Knowledge Park- I, Kasna Road, Greater Noida, Gautam Budh Nagar- 201310, Uttar Pradesh, INDIA.

Phone no: 09999427794

Email: rahulkcsji@gmail.com

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## INTRODUCTION

Epilepsy is a neurological disorder associated and characterized by paroxysmal, excessive and hyper synchronous discharge from large number of neurons. Despite the optimal use of available synthetic antiepileptic drugs (AEDs), many patients failed to experience seizure control and others do so only at the expense of significant toxicity that range in severity from minimal brain impairment to death, aplastic anemia, degenerative/degradative effects on some essential biochemicals on prolonged use and hepatic failure.<sup>1</sup> It is estimated that available medication controls seizures in only 50 % of patients or decrease incidence in only 75 % of patients. *Acorus calamus* Linn. (Araceae) commonly known as sweet flag is an ancient and time tested Ayurvedic herb prescribed as Medhya Rasayana for treatment of epilepsy, aphrodisiac and other brain related disorders. The reported uses of *Acorus calamus* are anticonvulsant,<sup>2</sup> antispasmodic<sup>3,4</sup> neuroprotective, antiulcerogenic,<sup>5</sup> antimicrobial<sup>6,7</sup> anti-inflammatory,<sup>8,9</sup> antioxidant and anticholinesterase,<sup>10</sup> antitumor<sup>11</sup> and hepatoprotective.<sup>12</sup> The rhizomes possessing sweet aromatic odour is reported to have strong anticonvulsant effects. The volatile oil from rhizomes of *Acorus calamus* was reported to contain the active anticonvulsant compound  $\beta$ -Asarone in significant amounts.<sup>13,14</sup> Methanolic extract of *Acorus calamus* was also reported to possess significant anti-seizure effect in subcutaneous pentylenetetrazol (scPTZ) induced convulsion model when administered orally in the doses of 100 and 200 mg/kg. It was also found to increase the latency period in mice significantly in scPTZ model.<sup>2</sup> The ayurvedic literature *Sharangdhara Samhita* has also highlighted the concept of polyherbalism to achieve greater therapeutic efficacy. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects. When multiple

herbs are combined in a particular ratio, it will give a better therapeutic effect and reduce the toxicity.<sup>15</sup> Bramhi Ghrita, a polyherbal formulation containing *Bacopa monnieri*, *Evolvulus alsinoides*, *Acorus calamus*, *Saussurea lappa* and cow's ghee was also reported to show anti-epileptic effects in MES and scPTZ models. It has also reduced alertness, spontaneous locomotor activity and reactivity. The formulation was also reported to potentiate Phenobarbital induced sleep and increases the pain threshold while antagonized the behavioural effects of d-amphetamine.<sup>16</sup> Sarasvata churna is an Ayurvedic formulation and as mentioned by *Bhavaprakasha* and Ayurvedic Formulary of India (AFI), is a compound Ayurvedic formulation containing *Saussurea lappa*, *Withania somnifera*, *Rock salt*, *Carum roxburghianum*, *Cuminum cyminum*, *Carum carvi*, *Piper longum*, *Piper nigrum*, *Zingiber officinale*, *Cissampelos pareira*, *Convolvulus pluricaulis*, *Acorus calamus* and *Bacopa monnieri*. Sarasvata churna ingredient herbs are prescribed in combination or individually for prevention of seizures and treatment of epileptic patients in a long run.<sup>15</sup> Mentat syrup, well prescribed polyherbal formulation has also shown a vital role in prognosis and management of children with febrile convulsions. It mainly contains herbs such as *Bacopa monnieri*, *Nardostachys jatamansi*, *Centella asiatica*, *Acorus calamus* and *Prunus amygdalus* and exhibited a significant improvement in children with febrile seizures.<sup>17</sup> *Acorus calamus* has also shown potent synergistic effects on anticonvulsant properties of prototype drugs Phenytoin and Phenobarbital and at a dose of 185 mg/kg it reduced the ED<sub>50</sub> of Phenytoin and Phenobarbital from 13.5 mg/kg to 9.25 mg/kg and 8 mg/kg to 5 mg/kg respectively. It also displayed significant increase in the antiepileptic activity of Phenytoin and Phenobarbital in the sub-effective dosage of 10 mg/kg and 2 mg/

kg respectively.<sup>18</sup> *Acorus calamus* also reported to prevent ferric chloride induced epileptogenesis in the dose of 200 mg/kg. It has also shown a significant decrease in the activity of superoxide dismutase and catalase enzymes with a decrease in the lipid peroxidation in cerebral cortex, thus indicating a potent anticonvulsant profile.<sup>19</sup>

In the present study, chloroform extract containing  $\beta$ -Asarone was evaluated for its anticonvulsant activity alone and in combination with methanolic extract of *Allium cepa* Linn. Which.<sup>20,21</sup> is supposed to possess antioxidant potential at varying doses using Isoniazid (INH) induced convulsion model.<sup>22</sup> Study was aimed to evaluate the effects of combinational therapy of an antioxidant herb over an anticonvulsant herb in order to establish the benefits of Polyherbalism in epilepsy treatment.

## MATERIALS AND METHODS

### Plant material

The plant material (rhizomes) of *Acorus calamus* was purchased from NC Herbs suppliers Barraut, Uttar Pradesh. Identification and taxonomical authentication was done by Dr. H.B. Singh, Head, Raw Materials Herbarium & Museum (RHMD) at National Institute of Science Communication and Information Resources, New Delhi (NISCAIR). The voucher specimen no. of authenticated plant material NISCAIR/RHMD/Consult/-2010-11/1587/185. Rhizomes are also preserved in the departmental museum of Ram-Eesh Institute of Vocational and Technical Education, Greater Noida. *Allium cepa* bulbs were purchased from NC Herbs suppliers, Barraut, Uttar Pradesh, self-authenticated and dried in shade.

**Drugs and Chemicals:** Sodium valproate (ENCORATE, Sun Pharma) and Isoniazid (LUP-INH, Lupin Ltd.) dissolved in normal saline were prepared on the day of the experimentation.

**Preparation of chloroform extract of *acorus calamus* and methanolic extract of *Allium cepa*:** The rhizomes of *Acorus calamus* were size reduced and extracted with chloroform in Soxhlet apparatus. The extract was concentrated in vacuum using rotary evaporator. The shade dried bulbs of *Allium cepa* were chopped so as to obtain maximum surface area and extracted with methanol using Soxhlet apparatus. The extracts were concentrated under vacuum and stored in refrigerator.

**Preparation of test drugs for Anticonvulsant screening:** The chloroform extract containing  $\beta$ -Asarone (liquid) (CBA, 50, 100, 150 mg/kg) and different dose combinations with methanolic extracts (solid) of *Allium Allium cepa* (CBAC) were prepared in normal saline and used for pharmacological evaluation.

### Experimental animals

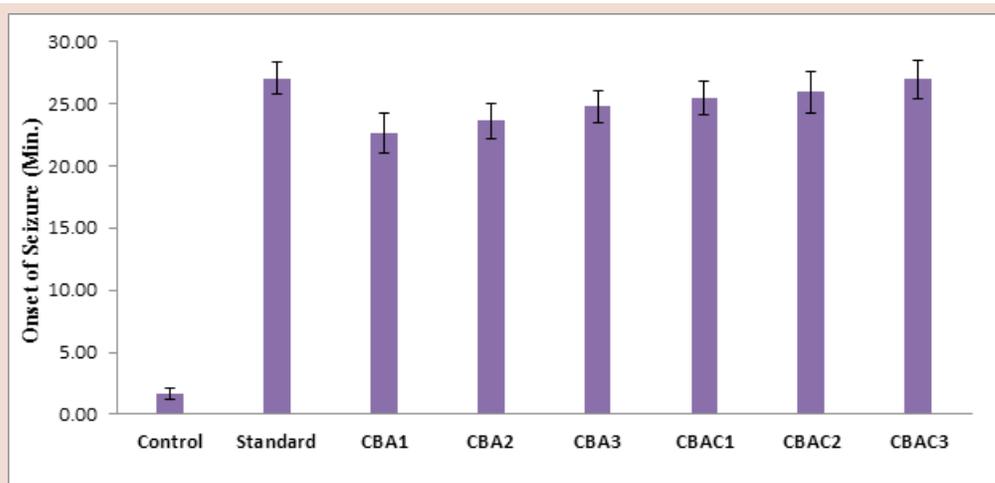
Swiss albino mice of either sex, weighing between 25-40 g were used in this study. All animal experiments were carried out in the CPCSEA approved laboratory (Reg. No. 385) under standard conditions. Animals were kept in group of 6 per cage at controlled temperature and humidity. Animals received standard diet and water *ad libitum*.

### Experimental design<sup>22</sup>

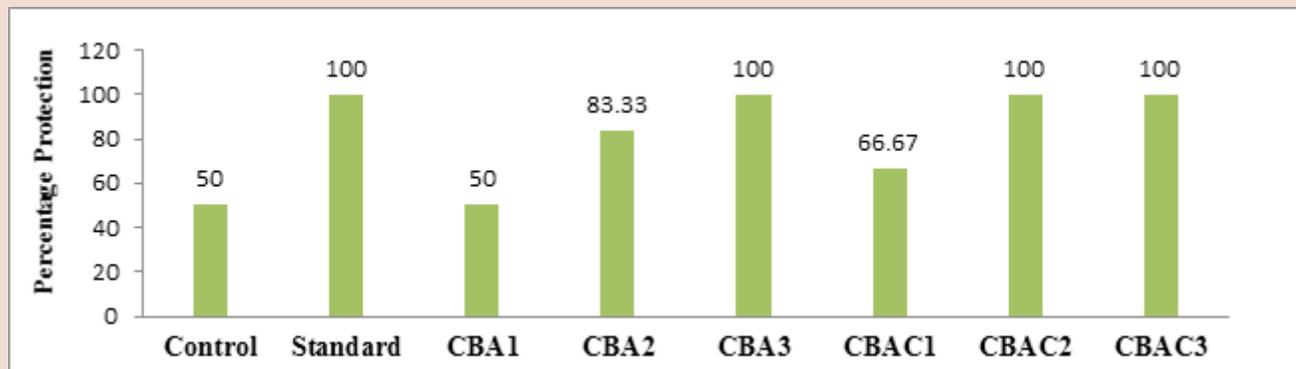
- Group I (n=6), Control animals (normal saline treated) - Control
- Group II (n=6), Animals treated with standard drug Sodium valproate (300mg/kg) in saline- Standard
- Group III (n=6), Animals treated with chloroform extract of Chloroform extract containing  $\beta$ -Asarone (50 mg/kg) in saline - CBA1
- Group IV (n=6), Animals treated with chloroform extract of Chloroform extract containing  $\beta$ -Asarone (100 mg/kg) in saline - CBA2
- Group V (n=6), Animals treated with chloroform extract of Chloroform extract containing  $\beta$ -Asarone (150 mg/kg) in saline - CBA3
- Group VI (n=6), Animals treated with chloroform extract containing  $\beta$ -Asarone (50 mg/kg) and methanolic extract of *Allium cepa* (100 mg/kg) in saline -CBAC1
- Group VII (n=6), Animals treated with chloroform extract containing  $\beta$ -Asarone (50mg/kg) and methanolic extract of *Allium cepa* (200mg/kg) in saline -CBAC2
- Group VIII (n=6), Animals treated with chloroform extract containing  $\beta$ -Asarone (50mg/kg) and methanolic extract of *Allium cepa* (300mg/kg) in saline -CBAC3

### Dose treatment

The different groups of animals were treated with varying doses of test and standard drugs as per the experimental design. Sodium valproate (300 mg/kg) was administered orally and INH (150mg/kg) was admin-



**Figure 1:** Effects of *Allium cepa* on the Anticonvulsant property of chloroform extract containing  $\beta$ -Asarone against INH induced seizures in mice.



**Figure 2:** Percentage protection against death in different treatments using INH induced seizures model in mice

Note:

- a. CBA= Chloroform extract containing  $\beta$ -Asarone at varying concentrations.  
 b. CBAC= Combination of Methanolic extract of *Allium cepa* (MAC) and CBA at varying concentrations.

istered intraperitoneally (i.p.). The animals were observed for time of onset of seizures and deaths. The observations for onset of seizure were presented in Figure 1 whereas observations for percentage protection against death were presented in Figure 2.

## RESULTS AND DISCUSSIONS

The chloroform extract of *Acorus calamus* containing  $\beta$ -Asarone as the major ingredient at a dose of 50, 100 and 150mg/kg have shown dose dependent protection of 50%, 83.33% and 100% respectively against death in the experimental mice. There were significant reduction in number and severity of seizures as the dose of CBA increases from 50mg/kg to 150mg/kg. The mean time for onset of seizure was also increased from 22.66 minutes in 50mg/kg to 23.61 minutes in 100mg/kg and 24.78 minutes in 150mg/kg dose of CBA indicating dose dependent protection and delay in time for seizure onset.

In combination with methanolic extract of *Allium cepa* (MAC), CBA in mice have shown increased time for seizure onset and less degree of severity of seizures as compared to CBA alone indicating the antioxidant benefits of *Allium cepa* over anticonvulsant activity of CBA. As the concentration of MAC increases from 100mg/kg to 200mg/kg while keeping the CBA at 50mg/kg, the percentage protection against death in animals increased from 66.67% to 100%. A 300mg/kg dose of MAC with 50mg/kg CBA offered significant protection, decreased severity and increased onset time for seizure. The mean time for seizure onset in combinational therapies (CBAC) has also revealed the antioxidant effects of *Allium cepa* on anticonvulsant activity of CAB. The mean seizure onset time in CBAC1 was 25.44 minutes whereas it was 22.66 minutes in CBA at 50mg/kg dose. The mean time increase to 25.94 minutes in CBAC2 and 26.94 in CBAC3 indicating the dose dependant delay in seizure onset.

The observations from combination therapies have shown significant protection in animals not only by delaying the mean seizure onset time but have also reduced the severity of seizures. The antioxidant potential of *Allium cepa* ameliorated the anticonvulsant potential of chloroform extract of *Acorus calamus* containing  $\beta$ -Asarone. The results have shown comparable antiepileptic potential to that of standard drug Sodium valproate.

## CONCLUSION

*Allium cepa* have tremendously ameliorated the antiepileptic potential of chloroform extract containing  $\beta$ -Asarone as major ingredient. This kind

of combination therapy not only increases protection but also reduces high degree of toxicities. Also, being naturally occurring ingredients these can be better option for synthetic antiepileptic drugs which are associated with degenerative effects and toxicities.

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## CONFLICT OF INTEREST

None.

## ABBREVIATION USED

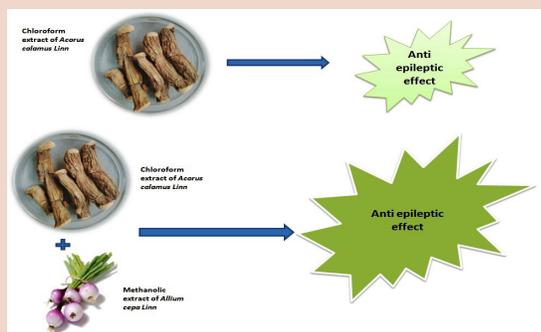
**CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **CBAC:** Combination of Methanolic extract of *Allium cepa* and Chloroform extract of *Acorus calamus* containing  $\beta$ -Asarone; **CBA:** Chloroform extract of *Acorus calamus* containing  $\beta$ -Asarone; **MAC:** Methanolic extract of *Allium cepa*; **INH:** Isoniazid; **NISCAIR:** National Institute of Science Communication and Information Resources; **RHMD:** Raw Materials and Herbarium Department; **ED:** Effective dose; **scPTZ:** Subcutaneously Pentylenetetrazol; **MES:** Maximal Electroshock; **AFI:** Ayurvedic Pharmacopoeia of India; **AED:** Anti-Epileptic Drugs.

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### PICTORIAL ABSTRACT



### ABOUT AUTHOR



**Rahul Kaushik** did his B.Pharm and M.Pharm (Pharmacognosy) from Dr. APJ Abdul Kalam Technical University, Lucknow. He is working in the Ram-Eesh Institute of Vocational & Technical Education, Greater Noida as Assistant Professor. He is also pursuing PhD from Dr. APJ Abdul Kalam Technical University, Lucknow. With over 6 Years of Academic and Industrial experience he has authored more than 10 research and review articles in International and National journals and attended more than 15 National conferences and seminars. His area of interest is Standardization of Herbal drugs, Chromatography, Phytochemistry and Herbal Anticonvulsants.



**Prof. (Dr.) Jainendra Jain** did M.Pharm from Banaras Hindu University Varanasi and Ph.D from Uttar Pradesh Technical University Lucknow. Currently he is associated with Ram-Eesh Institute of Vocational and Technical, Greater Noida as Principal. He has to his credit 37 research papers in National and International journals of high repute and also presented 29 poster presentations in various National and International conferences. He has guided more than 20 UG, 12 PG and 7 PhD scholars. He is recipient of many grants from All India Council of Technical Education (AICTE) Govt. of India, Department of Science and Technology, Dr. APJ Abdul Kalam Technical University and so on. He has successfully organized many National level workshops and seminars. He has keen interest in designing and development of both herbal and synthetic Anticonvulsants.



**Dr. Lubhan Singh** secured Gold medal in his M.Pharm (Pharmacology) from BPUT Rourkela, Orissa and Ph.D. from Uttarakhand Technical University, Dehradun. He is currently associated with Ram-Eesh Institute of Vocational and Technical, Greater Noida as Associate Professor and Head of Pharmacology Department. He has to his credit more than 35 research and review articles in National and International journals and has presented more than 50 posters in various National and International conferences and seminars. He has filed 01 patent and guided more than 100 UG and 10 PG students. He has coordinated 04 National conferences and seminars. He has received seminar grant from SERB. He has interest in CNS acting molecules.

### SUMMARY

- *Acorus calamus* possess anticonvulsant activity in MES and scPTZ models.
- *Allium cepa* possess Antioxidant potential.
- Combination of Anticonvulsant activity of *Acorus calamus* and Antioxidant potential of *Allium cepa* when screened in INH induced convulsion model resulted in good protection of animals against seizures even at low anticonvulsant dose of *Acorus calamus*.
- *Allium cepa* ameliorated the anticonvulsant effect of *Acorus calamus*.