

REVERSAL OF PHENYTOIN-INDUCED COGNITIVE IMPAIRMENT BY ACORUS CALAMUS IN MICE

YENDE, S. R., HARLE, U. N.,[?] BORE, V. V., BANSOD, M. S. AND PATIL, M. N.

Department of Pharmacology, AISSMS College of Pharmacy, Kennedy road, Pune
411001, India. E. mail: unharle@yahoo.co.in

Received: November 17, 2008; Accepted: December 15, 2008

Abstract: *The study deals with the investigation of the effect of methanolic extract of Acorus calamus rhizomes on phenytoin induced cognitive impairment in mice using passive-avoidance task, locomotor activity and acetylcholinesterase enzyme estimation. The regular administration of Phenytoin (75 mg/kg) showed cognitive impairment in passive avoidance task method by decreasing Step-Down Latency (SDL) and increase in Time Spent in the shock Zone (TSZ) significantly at 2 h and 24 h post-training. Acorus calamus (250 mg/kg) failed to produce any significant change in cognition. However, when given in combination with phenytoin, significantly increased SDL and decreased TSZ at 2 h and 24 h post-training when compared with phenytoin alone treated group. Acorus calamus showed significantly decreased locomotor activity unlike PHT and the combination of AC and PHT. The chronic oral administration of phenytoin significantly raised AChE level in the brain. Administration of Acorus calamus alone as well as in combination with PHT showed significant decrease in AChE level. Thus, this study provides evidence of the importance of Acorus calamus as adjuvant in antiepileptic therapy to reduce associated cognitive impairments.*

Key words: *Acorus calamus*, Phenytoin, Cognitive impairment, Acetylcholinesterase.

INTRODUCTION

Epileptic seizures are etiological deforms of both morphological and functional changes within the brain imparting cognitive and neuropsychological alterations. Antiepileptic drugs are intended to reduce seizure frequency and severity within the framework of safety. However, regular treatment with most antiepileptic conventional drugs may produce profound side-effects like cognitive impairment (i.e. memory attention, mental speed and learning) [1]. Further, most of the antiepileptic drugs have some neurotoxic effects and cognitive deficits which diminish their clinical utility in current medicinal practices [2,3]. Phenytoin (PHT) is amongst the anticonvulsants which is widely used and known to adversely affect learning and memory [4,5] by increasing the brain AChE levels [6]. Neuroprotective

agents are known to correct some of the observed cognitive impairments.

Acorus calamus Linn. (AC) (Family: Araceae), a semi aquatic perennial shrub commonly known as 'Sweet Flag', is recognised for its neuroprotective property in Auyurvedic literature and practice [7,8]. AC is indigenous to India, North America and Europe; preferably grown in shallow water or in a very moist loamy soil [9]. Its roots and rhizomes are commonly used in classical medicinal practice. The prominent chemical constituents of AC are α -asarone and β -asarone. It also contains amyralcohol, eugenol, calamenol, α -pinene (volatile oil), acorine (glycoside), acoretine (bitter principle), starch and tannin [10,11]. Furthermore, anticonvulsant activity of AC has been reported in rats [12,13].

Since AC has neuroprotective action, its significance in antiepileptic therapy of phenytoin was evaluated for possible beneficial effect on cognitive function. Further, this study was designed to evaluate the effect of AC for possible neuroprotection action against cognitive impairment caused by phenytoin.

MATERIALS AND METHODS

Animals: Swiss male albino mice (20-25 g) were housed in polypropylene cages at 25 ± 2 °C with a natural light-dark cycle and maintained on a daily scheduled of standard laboratory diet. Drinking water was supplied *ad libitum*. The experiments were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and approved by Institutional Animal Ethical Committee (IAEC) (Approval Number- CPCSEA/IAEC/PC-07/09-2K7).

Plant extract: Standard hydroalcoholic extract of rhizomes of *Acorus calamus* was gifted by Chemiloids, Vijaywada (Product Code- C/PCP/ACCA-01; Batch Number- L7091264). From extract β -asarone content was eliminated by successive extraction wherein, 100 g of hydroalcoholic extract was taken in 250 ml beaker, 100 ml of methanol was added and refluxed in water bath for 15 min; further, cooled and filtered through Whatman filter paper. The filtrate was dried and again extracted with methanol twice. The content of β -asarone was found to be decreased by HPTLC (data not shown).

Drugs and chemicals: Phenytoin (PHT) was procured from Anglo-French Ltd., (Banglore) as gift sample. 5,5-Dithiobisnitrobenzoic acid (DTNB) and Acetylthiocholine Iodide were procured from Ozone International, (Mumbai) and S. D. Fine-Chem Ltd., (Mumbai) respectively.

Experimental setup: Animals were divided into four groups. Each group contained six animals.

Group I: Vehicle treated.

Group II: PHT; Oral administration of phenytoin (75 mg/kg body weight/day) for 14 days.

Group III: AC; Oral administration of *Acorus calamus* (250 mg/kg body weight/day) for 7 days.

Group IV: AC + PHT; Oral administration of *Acorus*

calamus (250 mg/kg body weight/day) followed by Phenytoin (75 mg/kg body weight/day). PHT was administered for 14 days and AC (250 mg/kg, po) was administered for 7 days, which was added during the second week of treatment with PHT. As β -asarone is reported for carcinogenicity [14], so in this study, successive methanolic extract of AC was used to decrease its content [15].

Our preliminary study revealed that AC (250 mg/kg, po) has significant anticonvulsant and neuroprotective activity (data is not shown). The PHT (75 mg/kg, po) significantly impaired learning and memory [5]. Hence, the doses AC (250 mg/kg, po) and PHT (75 mg/kgpo) were selected. All doses were administered as oral suspension in distilled water using 1 % gum acacia using oral gavage.

Passive avoidance task: Passive avoidance task (PA) is a widely used method for screening drugs affecting cognitive impairment. The modified method of Papazova et al. [16] was employed for cognitive impairment test. In this method a continuous avoidance apparatus (Techno, Lucknow) with an inverted petridish placed in the centre of the grid floor serving as the shock-free zone (SFZ), was employed and followed by shock free interval of 60 seconds. The punishment was given to mice by electric shock (5mA, 20V, AC) through the grid floor on stepping down from the SFZ. The five trials were given to every mouse in different groups to avoid punishment (remain on SFZ). Animals not meeting these criteria of learning in five trials were rejected. During this experiment, observations were made for retention for maximum 300 s at 2 h and 24 h post-training. The retention parameters were noted as Step-Down Latency (SDL) in seconds and Time Spent in the shock Zone (TSZ) in seconds [17].

Locomotor activity (LA): The locomotor activity was assessed with Actophotometer (Space lab, Nashik). Photocells were located on the wall directly opposite to each photo-beam and connected to the Digiscan analyzer that records the number of beam breaks due to movement of the animal and display digitally. Interruption of one beam was recorded as one activity score [18]. After administration of drugs each mouse was placed individually in the Actophotometer for 5 min. and basal activity score was recorded.

Estimation of brain AChE activity: The whole

brain acetylcholine esterase activity was measured using Ellman et al. method [19]. This was measured on the basis of formation of yellow color due to the reaction of thiocholine with dithiobisnitrobenzoic ions. The rate of production of thiocholine as acetylthiocholine iodide in the presence of tissue enzyme was measured using spectrophotometer.

In this method, one hour after the last dose, brains were rapidly removed, weighed and homogenized in phosphate buffer (pH 7.2, 0.1 M) using tissue homogenizer to prepare a suspension of 20 mg of tissue per ml. This suspension was then centrifuged at 3000 rpm for 3-5 min at 10 °C. A 0.4 ml aliquot, 100 µl of dithiobisnitrobenzoic acid (DTNB) and 2.6 ml of phosphate buffer was vortex and absorbance was measured by UV spectrophotometer at 412 nm till absorbance became stable. This stable absorbance was set to zero and 20 µl of acetylthiocholine iodide (substrate) was added and changes in absorbance per minute were recorded at 412 nm for 10 minutes. The mean change in absorbance per minute was used for calculation and the rate of enzyme activity in µmol of acetylthiocholine iodide hydrolyzed /min/g of tissue was calculated.

Statistics: Results were expressed as Mean ± SD. The data was analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test and $P < 0.05$ was considered as statistically significant.

RESULTS

Effect of *Acorus calamus* extract on passive avoidance task: The regular administration of PHT (75 mg/kg) significantly ($P < 0.01$) decreased SDL at 2 h and 24 h post-training when compared with vehicle treated group. However, constant administration of AC (250 mg/kg) and the combination of AC (250 mg/kg) with PHT (75 mg/kg) significantly increased SDL ($P < 0.01$) at 2 h and 24 h post-training when compared with PHT treated group (Fig. 1).

The TSZ increased significantly by continuous

Table 1: Effect of phenytoin and *Acorus calamus* extract and their combination on locomotor activity. PHT, Phenytoin; AC, *Acorus calamus* extract. The results were expressed as Mean ± SD. (n=5), * $P < 0.05$ significantly differ from vehicle.

Treatment	Locomotor activity score (Mean±SD)
Vehicle treated	82.04 ± 5.05
PHT(75 mg/kg)	99.86 ± 18.05
AC (250 mg/kg)	49.12 ± 23.41 *
AC+PHT	55.37 ± 19.87

Table 2: Effect of phenytoin and *Acorus calamus* extract and their combination on brain Acetylcholine esterase (AChE) activity. PHT, Phenytoin; AC, *Acorus calamus* extract. The results were expressed as Mean ± SD.(n=5); a** $P < 0.01$, significantly differ from vehicle; b** $P < 0.01$, significantly differ from Phenytoin.

Treatments	AChE (µ mol) (Mean±SD)
Vehicle treated	1.25 ± 0.028
PHT (75 mg/kg)	1.55 ± 0.050 a**
AC (250 mg/kg)	0.95±0.041 a** b**
AC + PHT	1.19 + 0.074 b**

administration of PHT at 2 h and 24 h post-training when compared with vehicle treated group. The TSZ was found to be decreased by AC as well as its combination with PHT at 2 h and 24 h post-training when compared with PHT treated group (Fig. 2).

Effect of *Acorus calamus* extract on locomotor Activity: A regular administration of PHT showed increase in locomotor activity which was not statistically significant while AC showed significant decrease in locomotor activity. However, the combination of AC and PHT showed no significant effect on locomotor activity (Table 1).

Effect of *Acorus calamus* extract on brain AChE activity: The constant administration of PHT demonstrated a significant rise in brain AChE activity, while AC significantly decreased the enzyme level as compared to vehicle. Administration of AC alone as well as in combination with PHT showed significant decrease in whole brain AChE level when compared with PHT treated group (Table 2).

DISCUSSION

The results revealed a significant increase in TSZ and significant decrease in SDL this shows that the chronic administration of PHT leads to impairment of cognitive function in passive avoidance task. While administration of AC alone and in combination with PHT significantly reversed PHT induced cognitive impairments.

Motor stimulation is considered to be an interfering factor in the evaluation of cognitive effects of phenytoin [20]. In the present study PHT increases the locomotor activity which is not statistically significant, while AC showed decrease in locomotor activity. The decrease in locomotor activity of AC does not influence the PA task because it is reported that parameters viz SDL and TSZ remained unaffected by the locomotor activity [21].

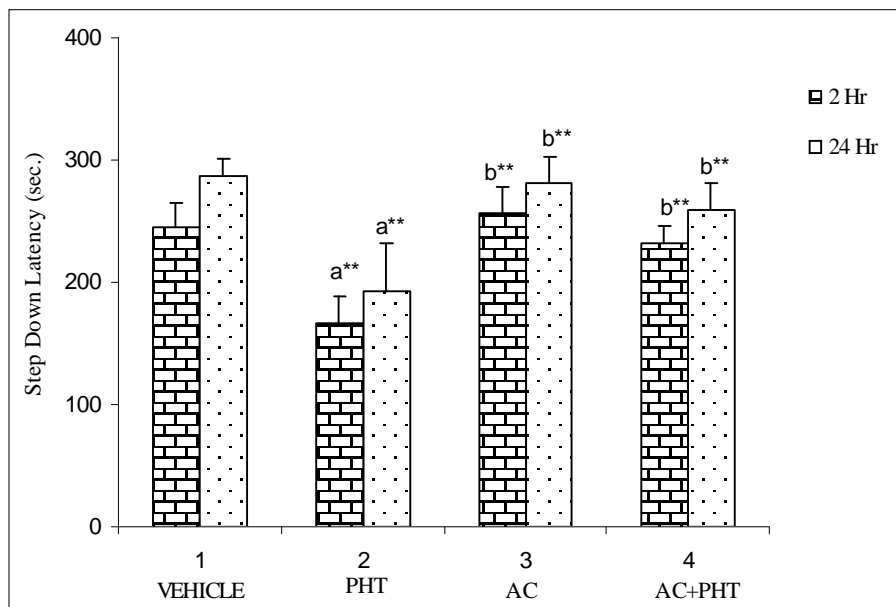


Fig. 1: Effect of Phenytoin (PHT 75 mg/kg), *Acorus calamus* extract (AC 250mg/kg) and their combination on SDL retention performance after 2 h and 24 h post training. (n=5); PHT, Phenytoin; AC, *Acorus calamus* extract. The results were expressed as Mean \pm SD. a** $P < 0.01$, significantly differ from vehicle; b** $P < 0.01$, significantly differ from Phenytoin.

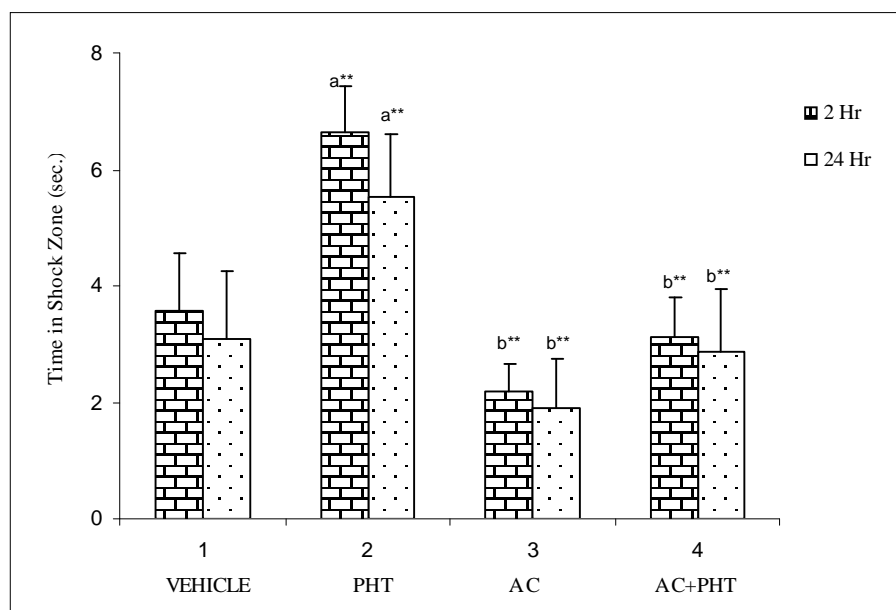


Fig. 2: Effect of Phenytoin (PHT 75mg/kg), *Acorus calamus* extract (AC 250mg/kg) and their combination on TSZ retention performance after 2 h and 24 h post training. (n=5); PHT, Phenytoin; AC, *Acorus calamus* extract. The results were expressed as Mean \pm SD. a** $P < 0.01$, significantly differ from vehicle; b** $P < 0.01$, significantly differ from Phenytoin.

The precise mechanism of AC showing the neuroprotection against the PHT induced cognitive impairment is not known but it is reported that the impairing effect of PHT on learning and memory has been attributed to alteration in cholinergic system [5]. Also PHT lowers brain acetylcholine (ACh) levels [22,23] and it significantly elevates the brain acetylcholine esterase activity [6]. In the present study it was found that AC alone decreases the brain

acetylcholine esterase level in control as well as in chronic PHT treated animals where enzyme level is elevated. Hence, these results emphasize that AC may increase neurotransmitter ACh, which may be responsible for the anticonvulsant and neuroprotective action of AC against PHT induced cognitive impairment. The α -asarone is reported to exhibit neuroprotective action through the blockade of N-methyl-D-aspartate receptor [24] and that α -asarone

inhibits AChE *in vitro* [25].

Study shows that the successive methanolic extract of rhizomes of AC reduces the cognitive impairment induced by PHT. This study is indicative of positive pharmacodynamic herb-drug interaction in order to minimize the cognitive adverse effect associated with antiepileptic therapy. Further investigations using a combination of AC and other antiepileptic drugs are warranted to explore the full potential of AC in correcting cognitive impairment associated with antiepileptic drugs.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. K.G. Bothara, Principal AISSMS College of Pharmacy, Pune for providing the necessary support; to Mr. Srinivasu, Chemiloids, Vijayawada, for providing herbal sample and to Mr. K. Hari, Anglo-French Drugs & Industries Ltd. Bangalore, for providing drug sample of Phenytoin.

REFERENCES

- [1] Vermeulen, J. and Aldenkamp, A.P.: *Epilepsy Res.*, 22: 65-95 (1995).
- [2] Trimble, M.R.: *Epilepsia.*, 28: 37-45 (1987).
- [3] Meador, K.J., Loring, D.W., Huh, K., Gallagher, B.B. and King, D.W.: *Neurology*, 40: 391- 394 (1990).
- [4] Aldenkamp, A.P., Alpherts, W.J., Diepman, L., Vantslot, B., Overweg, J. and Vermeulen, J.: *Epilepsy Res.*, 19: 37-43 (1994).
- [5] Sudha, S., Lakshmana, M.K. and Pradhan, N.: *Pharmacol. Biochem. Behav.*, 59: 119-124 (1995).
- [6] Shahid, M., Pillai, K.K. and Vohora, D.: *Indian J. Pharmacol.*, 36: 20- 24 (2004).
- [7] Shukla, P.K., Khanna, V.K., Ali, M.M., Khan, M.Y., Maurya, R., Handa, S.S. and Srimal, R.C.: *Phytother Res.*, 16: 256 - 260 (2002).
- [8] Shukla, P.K., Khanna, V.K., Ali, M.M., Khan, M.Y., Maurya, R. and Srimal, R.C.: *Hum. Exp. Toxicol.*, 25: 187- 194 (2006).
- [9] Nadkarni, K.M.: *Indian Materia Medica*. Popular prakashan Ltd, Vol - I, pp 35-37, Bombay (1998).
- [10] The Ayurvedic pharmacopoeia of India. Part-I, Vol-II, pp. 169- 170 (1999).
- [11] Prajapati, N.D., Purohit, S.S., Sharma, D.D. and Tarun, K.A.: *Handbook of Medicinal Plants*. Section II, Agrobios Jodhpur (india), (2003).
- [12] Hazra, R., Ray, K. and Guha, D.: *Biogenic Amines.*, 19: 177- 187 (2005).
- [13] Madan BR, Arora RB, Kapila K. *Int. Pharmacodyn. Ther.*, 124: 201-211 (1960).
- [14] Taylor, J.M., Jones, W.I., Hagan, E.C., Gross, M.A., Davis, D.A. and Cook, E.K.: *Toxicol. Appl. Pharmacol.*, 10: 405-412 (1967).
- [15] Mukherjee, P.K.: *Quality Control of Herbal Drugs*. Business Horizons Pharmaceutical publisher, New Delhi (2002).
- [16] Papazova, M.R., Bakarova, M.L. and Petkov, V.D.: *Pharmacol Biochem Behav.*, 49: 849- 852 (1994).
- [17] Vohora, D., Pal, S.N. and Pillai, K.K.: *J. Ethnopharmacol.*, 71: 383 - 390 (2000).
- [18] Luszczki, J.J.: *Indian J. Pharmacol.*, 36: 306-311 (2004).
- [19] Ellman, G.L., Courtney, K.D., Andres, V. and Featherstone, R.M.: *Biochem Pharmacol.*, 7: 88-98 (1961).
- [20] Dodrill, C.B. and Temkin, N.R.: *Epilepsia*, 30: 453 - 457 (1989).
- [21] Vohora, D. and Pillai, K.K.: *Indian J. Physiol. Pharmacol.*, 43: 63- 65 (1999).
- [22] Agarwal, S.L. and Bhargava, V.: *Indian J. Med. Res.*, 52: 1179- 1182 (1964).
- [23] Domino, E.F. and Olds, M.E.: *Psychopharmacology*, 23: 1- 16 (1972).
- [24] Cho, J.S., Kim, Y.H., Kong, J.Y., Yang, C.H. and Park, C.G.: *Life Sci.*, 71: 591- 599 (2002).
- [25] Mukherjee, P.K., Kumar, V., Mal, M. and Houghton, P.J.: *Planta Med.*, 73: 283- 285 (2007).