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Neuromodulatory effect of *Acrous calamus* leaves extract on dopaminergic system in mice

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Abstract : *Acorus calamus* (Family: *Araceae)*, available in India and many parts of world, found to have various Pharmacological activities such as analgesic, anti-convulsant, anti-spasmodic, anti-inflammatory, anti-bacterial, anti ulcer and cyto-protective. In present study, we have investigated effects of methanol (ACME) and acetone (ACAE) extract of *Acorus calamus* leaves against APM induced stereotypy and haloperidol induced catalepsy. APM induced stereotypy behavior, which reached peak at 15 min period. ACME (20 and 50 mg/Kg oral) administration significantly reversed stereotypy induced by APM. But ACAE at doses used (5, 20 and 50 mg/Kg oral) could not alter the stereotypy induced by APM. Whereas ACME (50 mg/Kg) and ACAE (20, 50 mg/Kg) administration significantly potentiated the haloperidol induced catalepsy in mice.
Key words: *Acorus calamus*; Catalepsy; stereotypy.

Introduction

Acorus calamus commonly known as sweet flag belongs to the family *Araceae*. This leaves are long, slender, sword-shaped and simple arising alternately from the horizontal rhizomes, which is widely available in continent of Europe, southern Russia, Northern Asia Minor, Southern Siberia, India, China and Japan[1].

The plant has been extensively investigated and a number of chemical constituents from the rhizomes, leave and roots of the plant have previously reported which includes β- Asarone (isoasarone) is usually the major constituent but is present in highly variable proportions and occasionally absent. α- Asarone, elemicine, cis-isoelemicine, cis and trans isoeugenol and their methyl ethers, camphene, P-cymene, β-gurjunene, α-selinene, β-cadinene, camphor,terpinen-4-ol, α-terpineol and α-calacorene, acorone, acrenone, acoragermacrone, 2-deca –4,7 dienol, shyobunones, linalool and preisocalamendiol are also present[2] .Acoradin, galangin, 2, 4, 5- trimethoxy benzaldehyde, 2,5- dimethoxybenzoquinone, calamendiol,spathulenol and sitosterol have been isolated from *Acorus calamus*[3]. The various pharmacological activities of *Acorus calamus* such as analgesic[4], anticonvulsant[5], antispasmodic[6] , anti-inflammatory[7], antibacterial[8] ,antiulcer and cytoprotective activity[9] was reported earlier. Most pharmacological activities of Acorus calamus was reported by using roots and rhizomes. Indian Acorus oil had shown sedative-tranquillizing action in rats, mice, cats, dogs and monkeys[10]. Roots and Rhizomes extracts of *Acorus calamus* Linn possess CNS depressant, tranquilizing, inhibiting the spontaneous motor activity[11]. But leaves extracts of *Acorus calamus* has shown analeptic/ CNS stimulant activity in *Drosophila melanogaster* as a model (US Patent no.6617491). In the present study, we investigated neuromodulatory activity of methanol and acetone extracts of *Acorus calamus* Linn. leaves on dopaminergic system by using apomorphine-induced stereotypy and haloperidol-induced catalepsy in mice.

Materials and Methods

Plant materials and extraction

The plant *Acorus calamus* (Family: *Araceae*) leaves was collected in Feb 2007 from the Kodaikanal Hills, Tamil Nadu, India. The plant material was taxonomically identified by the Botanical survey of India, Coimbatore, Tamilnadu, India and the voucher specimen ACL-20061

was retained in our laboratory for future reference. The dried powder material (500 g) of the leaves of *Acorus calamus* was ground and soaked in acetone and methanol, at room temperature. The dried leaves were soaked in a particular solvent for 3 days, each day the treated solvent being recovered and replaced with fresh solvents were then pooled together. The methanol and acetone extract was then distilled, evaporated and dried in vacuum. The resulted extract yield was 7.45% and the appearance of the extract was dry gum resin in nature. The chemical constituents of the extract were identified by qualitative analysis of chemical tests, which indicate the presence of volatile oil, tannins and terpenes.

Animals

Studies were carried out using Swiss albino mice (20–25 g). The animals were obtained from the animal house, KMCH College of Pharmacy, Coimbatore, India. The animals were grouped and housed in polyacrylic cages (38 x 23 x 10 cm) with not more than eight animals per cage and maintained under standard laboratory conditions (temperature 25 $\pm$ 2 ^{o}C) with dark and light cycle (14/10 hour). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water. The mice were acclimatized to laboratory condition for 10 days before commencement of experiment. All procedures described were reviewed and approved by the animals' ethical committee(IAEC) as per provisions of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), New Delhi, India .

Effect of *Acorus calamus* leaves extracts on apomorhine- induced stereotypy in mice

Measurement of stereotyped behavior was done as per method described below. The experimental animals were divided into four groups (n=6). In test groups (1,2 and 3) mice were pretreated with ACME and ACAE extracts at the dose of 5, 20, 50 mg/kg bw p.o. respectively 6 h prior to administration of apomorphine (APM) at the dose of 3mg /kg bw i.p. and mice were observed for stereotypy behavior for next 50 min. Separate vehicle control group of mice was also maintained to which only APM was administered. The intensity of stereotyped behavior was assessed at 5 minutes intervals throughout the duration of experiment.

Behavior was scored as described earlier[12,13].Score 0 (no change than control), 1 (discontinuous sniffing, constant exploratory activity), 2(continuous sniffing, periodic exploratory activity), 3(continuous sniffing, discontinuous biting, gnawing or licking .Very brief periods of locomotor activity) or 4 (continuous biting, gnawing or licking; no exploratory activity)

Effect of *Acorus calamus* leaves extracts on haloperidol-induced catalepsy in mice

In case of haloperidol (HP)-induced catalepsy, the experimental animals were divided similar to previous experiment and administered with HP at the dose of 0.1mg/kg bw i.p to all the group. All three groups except control were administerd with ACME and ACAE at the dose of 5, 20.50 mg/kg bw p.o respectively. The control group was administed only with vehicle. Catalepsy was scored in a manner similar to that described by Ahtee and Buncombe (1974). Animals were tested for the presence of catalepsy by placing both front paws on a 4 cm high wooden block, a cataleptic animal maintaining this position for a period of tune dependent upon the degree of catalepsy. If the animal maintained the imposed posture for at least 20 s it was said to be cataleptic and given one point. For every further 20 s it continued to maintain the cataleptic posture one extra point was given, thus the animal was given a score of 2 points if it maintained the posture for 40 s, 3 points for 60 s, and so on. The animals were tested for catalepsy 30 min after haloperidol treatment[14].

Statistical analysis

The Effect of *Acorus calamus* leaves extracts on APM-induced stereotypy and HP-induced catalepsy was expressed as mean score ±SEM for stereotypy and catalepsy respectively. Data was analyzed by Kruskal-Wallis Test on ranks followed by Dunn's test.

Results and Discussion

Anti-psychotic drugs like haloperidol and chlorpromazine (the so- called typical neuroleptics) induce abnormal motor behaviors in experimental animals and humans, including catalepsy in rats and mice[15] . Neuroleptic-induced catalepsy in rodents is a robust behavioral method for the study of nigrostriatal dopaminergic function and its modulation by other transmitter systems[15,16] it is generally accepted that dopaminergic system in the brain is important for the mediation for of drug induced stereotyped behavior. The nigrostriatal dopaminergic pathway has long been implicated in motor functioning[15]. Dopamine is present in the region of nucleus accumbens and is responsible for locomotor activity, while stereotypy is mediated by striatal dopaminergic neuron[15]. Stereotyped behavior may operate via a reciprocal balance between the dopaminergic and cholinergic systems, in favour of dopaminergic dominance.

In present study, we investigated effects of ACME and ACAE against APM induced stereotypy and HP induced catalepsy behaviour. APM directly activates dopamine receptors in the brain[17,18] and larger doses of the drug induced stereotyped behaviour (sniffing, licking and gnawing) [19]. The stimulant effect of high doses of APM is attributed to activation of post synaptic receptors in the CNS[19]. The behavioral responses observed in animals after administration of the dopamine agonist, APM are attributed to activation of D_1 and D_2 receptors[17,18]. Mesolimbic and nigrostriatal dopaminergic pathways may be important in the mediation of locomotor activity and stereotyped behaviours. Stereotyped behaviour is more closely associated with the caudate striatum area of

brain[20]. Acute treatment of alcoholic extracts (10, 25 and 50 mg/kg, i.p) of roots and rhizomes of *Acorus calamus* extracts antagonized spontaneous motor activity and also amphetamine-induced hyperactivity in mice. But chronic administration of ethanolic extract of Acorus calamus significantly increased dopamine level in the caudate nucleus and midbrain and decreased in the cerebellum[21]. It was reported pretreatment of Indian Acorus oil had a reserpine-like action in depleting rat brain of noradrenaline and serotonin[22]. Indian Acorus oil also had shown sedative-tranquillizing action in rats, mice, cats, dogs and monkeys. Doses of 25 and 50 mg/kg, i.p produced vomiting in cats, dogs[10]. These studies revealed the interaction of Acorus calamus on dopaminergic system in the brain.

Effect of *Acorus calamus* leaves extracts on APM-induced stereotypy

APM induced stereotypy behavior, which reached peak at 15 min period (Figure 1). ACME at the dose of 20 and 50 mg/kg bw p.o. administration significantly reversed stereotypy induced by APM. But ACAE at doses used 5, 20, 50 mg/kg bw p.o. could not alter the stereotypy induced by APM. Acute treatment of ACME, which influences the central dopaminergic mechanisms have been found to affect stereotyped behavior induced by APM and suggested to be modulator of dopaminergic neurons in nigro-striatal system.Dopamine (D_2) antagonist.

Effect of ACME and ACAE on HP- induced catalepsy

HP increases striatal dopamine release and induces catalepsy through it is actions on striatal dopaminergic system[23] and proved to be simple and reliable test for the investigation that involves D_2 receptor (Fischer et.al, 2002).ACME at the dose of 50 mg/kg bw p.o and ACAE at the doses of 20 and 50 mg/kg bw p.o. significantly potentiated the haloperidol induced catalepsy in mice (Table 1). This study further confirms the neuromodulatory effects of *Acorus calamus* leaves extracts on striatal dopaminergic system.

Conclusion

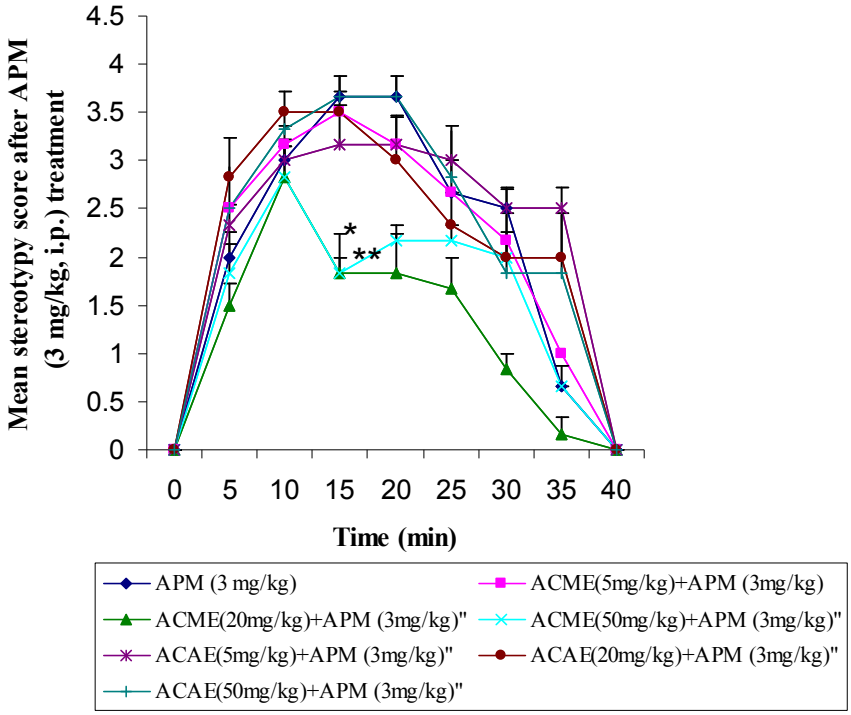
The effect of ACME and ACAE pretreatment at various doses against APM induced stereotyped behavior and HP induced catalepsy in mice was studied. ACME (20, 50 mg/kg bw p.o) significantly reversed stereotypy induced by APM, when administered 6 h prior to APM. But ACAE at doses used 5, 20, 50 mg/kg bw p.o. could not alter the stereotypy induced by APM. It is also found that ACME (50 mg/kg bw p.o.) and ACAE (20, 50 mg/kg bw p.o.) administration significantly potentiated the haloperidol induced catalepsy in mice. These results suggest that *Acorus calamus* leaves extracts exerts neuromodulatory effects on nigro-striatal dopaminergic system.

Table 1. Effect of *Acorus calamus* Leaves Extracts on Haloperidol-induced Catalepsy in Mice

Drugs	Dose(mg/kg, p.o.)	Catalepsy score after 30 min
HP	0.1	0.6667±0.3333
ACME+ HP	5.0	2.500±1.310
ACME+ HP	20	1.167±0.166
ACME+ HP	50	3.500±0.8851*
ACAE+ HP	5.0	2.667±0.5578
ACAE+ HP	20	3.667±0.8028*
ACAE HP	0	3.500±0.2236**

ANOVA Values: F (6,35)=2.729; p< 0.05;Values are mean± SEM of 6 animals a group.
***p<0.05, **p<0.01 as compared with haloperidol treated group.**

Figure 1.Effect of Acorus calamus leaves extract on apomorphine induced stereotypy in mice



Values are mean± SEM of 6 animals a group. *p<0.05, **p<0.01 as compared with apomorphine treated group at 15 min (where apomorphine showed peak stereotypy score)

References

1. Motley T. J.,The ethno botany of sweet flag, *Acorus calamus* (Araceae), Economic Botany, 1994,48(4),397.
2. Willamson, E. M., AND Evans F. J., Potter's new cyclopedia of botanical drugs and preparations . CW Daniels ,Saffron Walden. 1988.
3. Mazza G., Gas chromatographic and mass spectrometry studies of the constituents of the rhizome of *Acorus calamus* II, The volatile constituents of essential oil, Journal of chromatography, 1985, 328,179.
4. Menon M. K. and Dandiya P.C., The mechanism of tranquilizing action of asarone from Acorus calamus Linn, Journal of pharmacology, 1967, 19(3),170.
5. Narayan J., Pandit B.S.V. and Rangesh P.R.M.D., Clinical Experience of a compound Ayurvedic preparation on apasmara (epilepsy), Ayurveda Vignyana, 1987, 9(5),7.
6. Dey D. and Das M.N., Pharmacognosy of antidysenteric dugs of Indian medicine, Acta Botanica Indica, 1988,16,216.
7. Vohora S.B., Shah A.S., Shama A., Naqvi S.A.H., Dandiya P. C., Anti bacterial, anti pyretic analgesic and anti inflammatory studies on Acorus calamus Linn, Annals of the National Academy of Medical Science,1989, 25 (1),13.
8. Syed M., Riaz M., Chaudhari F.M., The anti bacterial activity of the essential oils of the Pakistani Acorus calamus, Callistemon lanceolatus and Laurus nobilis, Pakistan Journal of Science and Industrial Research, 1991,34(11),456.
9. Rafatullah S., Tariq M., Mossa J.S., Al- Yahaya M.A., Al – Said M.S., Ageel A.M., Anti-secretagogue, anti-ulcer and cytoprotective properties of *Acorus calamus* in mice, Fitoerapia, 1994, 65,19.
10. Dhalla N. S. and Bhattacharya I. C., Further studies on neuro pharmacological actions of Acorus oil, Arch.Int.pharmacodyn, 1968,172, 356-365.
11. Panchal G.M., Venkatakrishna- Bhatt H., Doctor R. B., Vajpayee S., Pharmacology of Acorus calmus Linn, Indian J Exp Biol., 1989 jun,27(6):561-7.
12. Battisti J. J., Shreffler C. B., Uretsky N. J. and Wallace L. J., NMDA antagonists block expression of sensitization of amphetamine and apomorphine induced stereotypy,Pharmacol.Biochem.Behav.,2000, 67:241-246.

13. Pandi V., Nagappa A. N. and Thakurdesai A., Effects of Losartan Potassium on Central Dopaminergic System in Mice, Journal of Pharmacology and Toxicology,2007,2: 551-558.
14. Ahteel L. and Buncombe G., Metoclopromide induces catalepsy and increases striatal homovanillic acid content in mice, Acta Pharmacol.toxicol, 1974, 35, 429-432.
15. Sanberg P.R., Bunsey M. D., Giordano M., and Norman A. B., The catalepsy test: its ups and downs. Behav. Neurosci., 1988,102:748-759.
16. Pires J. G., Silva S.R. and Futuro-Neto H. A., Effects of losartan on neuroleptic-induced catalepsy in mice. Brazilian, Journal of Medical and Biological Research, Revista Brasileira de pesquisas medicas e biologicas /sociedade Brasileira de Biofisica., 1996, 29:1045-1047.
17. Seeman P., Brain dopamine receptor, Pharmacol, Rev., 1980, 32:229-313.
18. Stoof J.C. and Kebabian J. W., Two dopamine receptor: Biochemistry physiology and pharmacology, Life sci.,1984,35:2281-2296.
19. Anden N.E., Rubenson A., Fuxe K. and Hokfelt., Evidence for dopamine receptor stimulation by apomorphine, J.pharm. pharmacol, 1967, 19:627-629.
20. Kelly P. H., Seviour P.W. and Iversen S.D., Amphetamine and Apomorphine response in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum,Brain Res., 1975,94:507-522.
21. Hazara R. and Guha D., Effect of chronic administration of Acorus calamus on electrical activity and regional monoamine levels in rat brain, Biogenic Amines, 2002, Vol 17, 161-169.
22. Malhotra C.L., Effect of hersaponin and acorus oil nor adrenaline and 5-hydroxy triptamine content of rat brain, J.Pharm.Pharmacol., 1961, 13.447.
23. Jaskiw G. E. and Bongiovani R., Brain tyrosine depletion attenuates haloperidol-induced striatal dopamine release in vivo and augments haloperidol -indcuced catalepsy in the rat, psychopharmacology,(Berl), 2004, 172:100-107.

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