

Depressant Effect of Trimyristin and Its Inhibition by Some Antidepressants in Mice

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Abstract

The depressant activity of trimyristin and its interaction with antidepressants like amitriptyline, imipramine, mianserin, and fluoxetine has been studied. In forced swim tests and tail suspension tests male albino mice were treated intraperitoneally with 10 mg/kg each of amitriptyline, imipramine, mianserin, and fluoxetine 30 min before vehicle or trimyristin (10 or 30 mg/kg i.p.). Some groups received trimyristin (10 or 30 mg/kg i.p.). In reserpine-induced hypothermia, the effect of trimyristin was observed on hypothermia induced by reserpine. In forced swim tests and tail suspension test, trimyristin increased the duration of immobility and pretreatment with antidepressants inhibited the effect of trimyristin. Trimyristin significantly lowered the rectal temperature and potentiated the reserpine-induced hypothermia. In the forced swim tests, the depressant effect of trimyristin was inhibited by prior administration of serotonin 5-HT_{2A} receptor antagonist, saproterelate suggesting involvement of serotonergic and noradrenergic mechanisms in the depressant action of trimyristin.

INTRODUCTION

Myristica fragrans Houtt (Myristicaceae), commonly known as nutmeg, is now cultivated in many tropical countries. A ligroin extract of *M. fragrans* caused significant increase in duration of sleep in chickens (Sherry et al., 1982). The hexane extract of nutmeg significantly inhibited drug metabolizing enzyme activity and showed prolongation of hexobarbital-induced sleep (Shin et al., 1988). Truitt et al. (1988) reported hallucinogenic activity of nutmeg. The ethanolic extract of *M. fragrans* demonstrated significant hypolipidemic effects and lowered the high-density lipoprotein and low-density lipoprotein ratio in rabbits. It also lowered the level of total cholesterol in the heart and liver (Sharma et al., 1995; Ram et al., 1966). The volatile oil of nutmeg was found to be effective as antioxidant (Kumarvelu et al., 1996). In addition to these properties, nutmeg is reported to possess stimulant, narcotic, carminative, astringent, aphrodisiac (Nadkarni, 1998), anti-thrombotic and anti-platelet aggregation (Olajide et al., 1999), antibacterial, antifungal (Orabi et al., 1991), anti-dysenteric (Gills, 1994), anti-inflammatory and analgesic activities (Ozaki et al., 1989; Olajide et al., 1999). Previous studies from our laboratory have shown sedative, analgesic, anticonvulsant, and anxiogenic actions of nutmeg. Trimyristin has been shown to be responsible for the anxiogenic activity (Sonavane et al., 2001, 2002 a,b). Since anxiety is often associated with depression (Nutt, 1999) and nutmeg increases hexobarbital-induced sleep, we studied the effect of trimyristin on reserpine-induced hypothermia in rats and behavioral despair induced by forced swimming and tail suspension in mice to study the possibility of developing depression with trimyristin. Several antidepressants, having different modes of action, were used in the present study to elucidate the probable mode of action. Since the serotonin 5-HT₂ receptor blocker has been tried in the care of depressed patients with anxiety symptoms (Fuller, 1991; Nutt, 1999), we have also studied the effect of selective 5-HT₂ antagonist, saproterelate on trimyristin induced depression.

MATERIALS AND METHODS

Animals

Albino mice (Swiss, 20-25g of either sex) were housed in groups of 6-8 per cage at the ambient temperature of $25\pm 1^{\circ}\text{C}$ and relative humidity of $50\pm 5\%$. A 12:12h light:dark cycle was followed during the experiments. The animals had free access to animal chow (Lipton, India) and water however, food was withheld overnight before the experiments. The experiments were performed between 10:00 and 15:00h. The Institutional Animal Ethical Committee approved the protocol.

Drugs

Imipramine, amitryptiline, and mianserin were gifts from Torrent Pharmaceuticals (Ahmedabad). Cadila Laboratories (Ahmedabad), supplied the fluoxetine. Saprorelate was a gift of Dr. R.K. Goyal, L.M. College of Pharmacy (Ahmedabad). The solvents used in the study were purchased from Modern Scientific Co. (Nashik). Trimyristin (TM) was isolated from the petroleum ether extract of *Myristica fragrans* seeds as described earlier by Sonavane et al. (2001). Trimyristin was dissolved in polyethylene glycol 400 for administration. The volume of injection was 0.1 ml per animal.

Effect on Reserpine-induced Hypothermia

Rats were divided into four groups of five each. Reserpine (3 mg/kg i.p.) was administered 30 min after vehicle or trimyristin (10 mg/kg i.p. each). In another set of experiments, one group received trimyristin alone and in the other group amitryptiline (10 mg/kg i.p.) was administered 30 min before trimyristin. The rectal temperature was measured using a telethermometer as described earlier by Turner (1972). The rectal temperature was recorded at 0, 30, 60, 90, 120, 150 and 180 min after reserpine or trimyristin.

Forced Swim Test

Mice were placed in a glass cylinder (40 x 18 cm) containing 10 cm water, maintained at 25°C . After 10 min the mice were removed and dried. The next day, the mice were treated intraperitoneally with imipramine, amitryptiline, fluoxetine and mianserin (10 mg/kg each) or saprorelate (2 mg/kg) 30 min before TM (10 and 30 mg/kg) or vehicle. Thirty min after administration of TM administration animals were gently put in water and the total duration of immobility was measured during 5 min test period, after discarding first 2 min since animals exhibited vigorous attempts to escape (Porsolt et al., 1977).

Tail Suspension Test

The tail suspension test was carried out as described earlier by Steru et al. (1985). The drugs were administered as described in the forced swimming test. The duration of immobility was measured during the test period of 8 min.

Statistical Analysis

All data were presented as mean \pm SEM. The Kruskal Wallis test was used for reserpine-induced hypothermia. The data obtained from other tests was analyzed by one way ANOVA followed by Dunnett's test. $P < 0.05$ was considered significant.

RESULTS

Reserpine-induced Hypothermia

Reserpine gradually induced hypothermia and maximum effect was observed 90 min after reserpine administration. Trimyristin also induced hypothermia and concurrent treatment with reserpine potentiated hypothermia. Amitriptyline inhibited trimyristin-induced hypothermia significantly. The observations are given in Fig. 1.

Forced Swimming Test

The total duration of immobility in the vehicle treated group was observed to be 75.4 ± 3.5 sec. TM, 10 mg/kg and 30 mg/kg increased the duration of immobility to 104.5 ± 1.98 sec and 204.5 ± 18.14 sec respectively. The amitriptyline, imipramine, mianserin and fluoxetine used *per se* reduced the duration of immobility and opposed the effect of BF significantly. Fluoxetine was found to be most effective in reducing the duration of immobility. The effect of trimyristin was also inhibited by saprorelate. The observations are given in Fig. 2.

Tail Suspension Test

The total duration of immobility in the vehicle treated group was observed to be 112.7 ± 1.97 sec. TM, 10 mg/kg and 30 mg/kg increased the duration of immobility to 226.5 ± 2.59 sec and 301.2 ± 4.87 sec respectively. The amitriptyline, imipramine, mianserin and fluoxetine used *per se* reduced the duration of immobility and opposed the effect of TM significantly. Mianserin was found to be most effective in reducing the duration of immobility. The observations are given in Fig. 3.

DISCUSSION

The outcome of the present study indicates that trimyristin induces depression in mice and several antidepressants have inhibited the effect of trimyristin. Reserpine and tetrabenazine have been used in the past to induce depression in laboratory animals. Since previous studies from this laboratory have reported anxiogenic action of *Myristica fragrans* seeds (Sonavane et al., 2002a) it was our endeavor to investigate the potential of *M. fragrans* to induce depression in mice. Reserpine and tetrabenazine reduces brain contents of both serotonin and noradrenaline and reduces locomotion. The depletion of serotonin and noradrenaline is very fast and weak antidepressants cannot inhibit their depressant activity. Though tricyclic antidepressants have been used for a long time, their mode of action is not yet clearly understood. The major metabolites of tricyclic antidepressants have considerable pharmacological activity and often differ from the parent drug in their noradrenaline/serotonin selectivity. Since noradrenaline and 5-HT have been implicated in the etiology of depression, decrease in the duration of immobility in forced swim test and tail suspension tests has been linked to the increased availability of these neurotransmitters at the post-synaptic receptor sites.

The induction of hypothermia by reserpine was found to be more prominent than trimyristin. The body temperature reduced further after conjoint treatment of trimyristin and reserpine and amitriptyline reversed the effect of trimyristin suggesting that trimyristin-induced depression can be another animal model to screen antidepressants.

Similar results were observed in the forced swim test, a well established animal model of depression. In this model the effect of trimyristin was maximally attenuated by fluoxetine. The tail suspension test has been considered refinement of the behavioral despair test. Steru et al. (1985) demonstrated that imipramine, desipramine, amitriptyline, and mianserin induced a dose-related reduction of immobility. Pal and Dandiya (1993) also demonstrated that the tail suspension test is more sensitive than the forced swim test. In the tail suspension test the maximum inhibition of trimyristin effect was exhibited by mianserin. Mianserin, an atypical antidepressant, which blocks α_2 , 5-HT₂ and H₁ histamine receptors, possibly increases noradrenaline release (Rang et al., 2002) and because of multiple action the effect of mianserin was better than the other drugs used in this study. Similar differential effects have also been noticed in different animal as well as human studies. Noradrenaline selective antidepressants have shown better efficacy than serotonin selective antidepressants in clinical trials (Dubuni et al., 1997). Saprorelate was found to be less active than mianserin indicating that neurotransmitter in addition to serotonin is involved in the depressant activity of trimyristin. Thus the present study concludes that trimyristin induces depression by a non-specific mechanism and nutmeg should be avoided in patients suffering from depression.

Literature Cited

- Dubini, A., Bosc, M. and Polin, V. 1997. Noradrenaline-selective versus serotonin-selective antidepressant therapy: differential effects on social functioning. *J. Psychopharmacol.* 11:S17-23.
- Fuller, R.W. 1991. Role of serotonin in therapy of depression and related disorders. *J. Clin. Psychiatry* 52:52-57.
- Gills, C.V. and Cox, P.A. 1994. Ethnobotany of nutmeg in the Spice Islands. *J. Ethnopharmacol.* 42:117-124.
- Kumarvelu, P., Subramanyam, S., Dakhinmurthy, D.P. and Devraj, N.S. 1996. The anti-oxidant effect of eugenol on CCl₄ induced erythrocyte damage in rats. *J. Nutrit. Biochem.* 7:23-28.
- Nadkarni, K.M. 1998. *Myristica fragrans*. p.830-834. In: *Indian Materia Medica*, 3rd Ed., Bombay Popular Prakashan, Bombay.
- Nutt, D.J. 1999. Care of depressed patients with anxiety symptoms. *J. Clin. Psychiatry* 60:23-27.
- Olajide, O.A., Ajayi, F.F., Ekhehar, A.I., Awe, S.O., Makhinde, J.M. and Alada, A.R.A. 1999. Biological effects of *Myristica fragrans* (nutmeg) extract. *Phytotherapy Res.* 13:344-345.
- Orabi, K.Y., Mossa, J.S. and El-Ferally, S. 1991. Isolation and characterization of two antimicrobial agents from mace (*Myristica fragrans*). *J. Nat. Product* 54:856-859.
- Ozaki, Y., Soedigo, S., Wattimena, Y.R. and Suganda, S.G. 1989. Anti-inflammatory effect of mace, aril, of *Myristica fragrans* Houtt. and its active principles. *Jpn. J. Pharmacol.* 49:155-163.
- Pal, S.N. and Dandiya, P.C. 1993. Comparative study of imipramine, maprotiline, fluvoxamine, trazodone and alprazolam in some animal models of depression. *Indian J. Pharmacol.* 25:204-208.
- Porsolt, R.D., Bertin, A. and Jalfre, M. 1977. Behavioral despair in mice: A primary screening test for antidepressants. *Arch. Int. Pharmacodyn.* 229:327-336.
- Ram, A., Lauria, P., Gupta, R. and Sharma, V.N. 1966. Hypolipidemic effect of *Myristica fragrans* fruit extract in rabbits. *J. Ethnopharmacol.* 55:49-53.
- Rang, H.P., Dalem, M.M. and Ritter, J.M. 2000. (Eds.) *Pharmacology*, Churchill Livingstone. London. 562-563p.
- Sharma, A., Mathur, R. and Dixit, V.P. 1995. Prevention of hypercholesterolemia and atherosclerosis in rabbits after supplementation of *Myristica fragrans* seeds extract. *Indian J. Physiol. Pharmacol.* 39:407-410.
- Sherry, C.J., Ray, L.E. and Herron, R.E. 1982. The pharmacological effect of ligroin extract of nutmeg. *J. Ethnopharmacol.* 6:61-66.
- Shin, K.H., Kin, O.N. and Woo, W.S. 1988. Isolation of hepatic drug metabolism inhibitors from the seeds of *Myristica fragrans*. *Arch. Pharmacol. Res.* 11:240-243.
- Sonavane, G.S., Sarveiya, V., Kasture, V.S. and Kasture, S.B. 2001. Behavioral actions of *Myristica fragrans* seeds. *Indian J. Pharmacol.* 33:417-424.
- Sonavane, G.S., Sarveiya, V.P., Kasture, V.S. and Kasture, S.B. 2002a. Anxiogenic activity of *Myristica fragrans* seeds. *Pharmacol., Biochem. Behav.* 71:239-244.
- Sonavane, G.S., Kasture, V.S. and Kasture, S.B. 2002b. Anticonvulsant and behavioral actions of *Myristica fragrans* seeds. *Indian J. Pharmacol.* 34:332-338.
- Steru, L., Chermat, R., Thierry, B. and Simon, P. 1985. Tail suspension test: A new method for screening antidepressants in mice. *Psychopharmacol.* 85:367-370.
- Truitt, E.B., Callway, E., Braude, M.C. and Krantz, J.C. 1988. The pharmacology of myristicin: A contribution to the psychopharmacology of nutmeg. *J. Neuropsychiatr.* 2:205-210.
- Turner, R.A. 1972. *Screening procedures in Pharmacology*, Academic Press, New York.

Figures

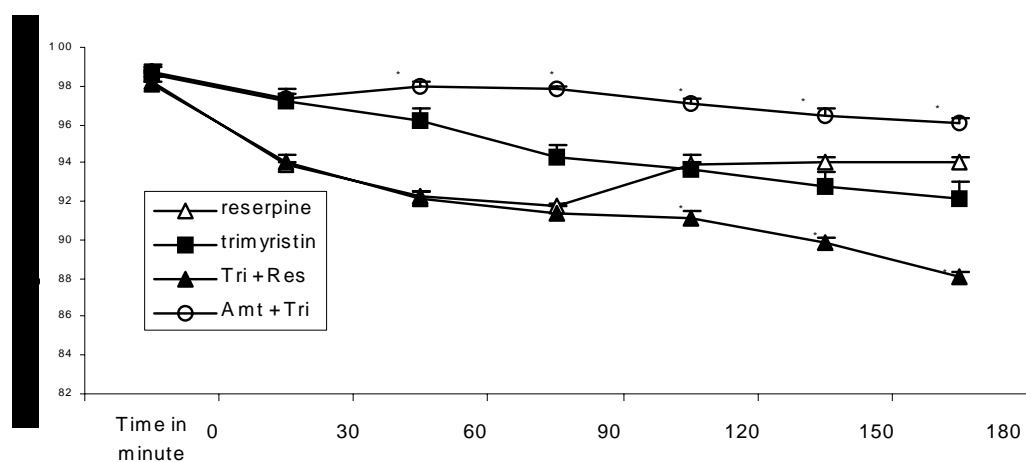


Fig. 1. Effect of trimyristin on rectal temperature in rats. * $P < 0.05$ compared to its respective control (Kruskal Wallis ANOVA).

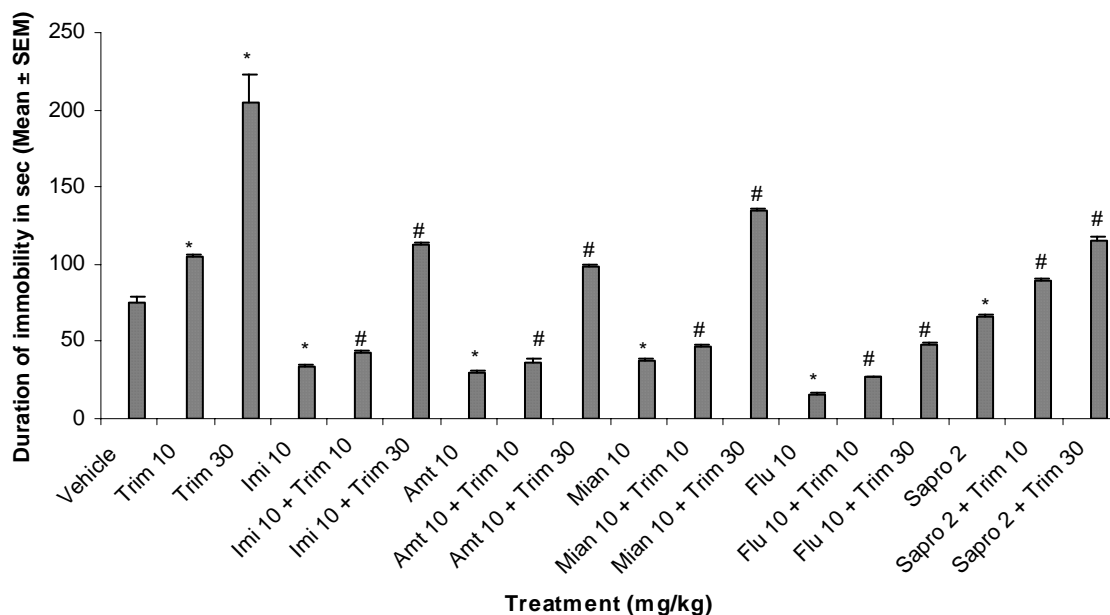


Fig. 2. Effect of trimyristin and antidepressants on duration of immobility in forced swim test in mice. $F = 103.59$, $P < 0.0001$. * $P < 0.05$ compared to the vehicle treated group and # $P < 0.05$ compared to Trim (10 or 30 mg/kg) (ANOVA followed by Dunnett's test).

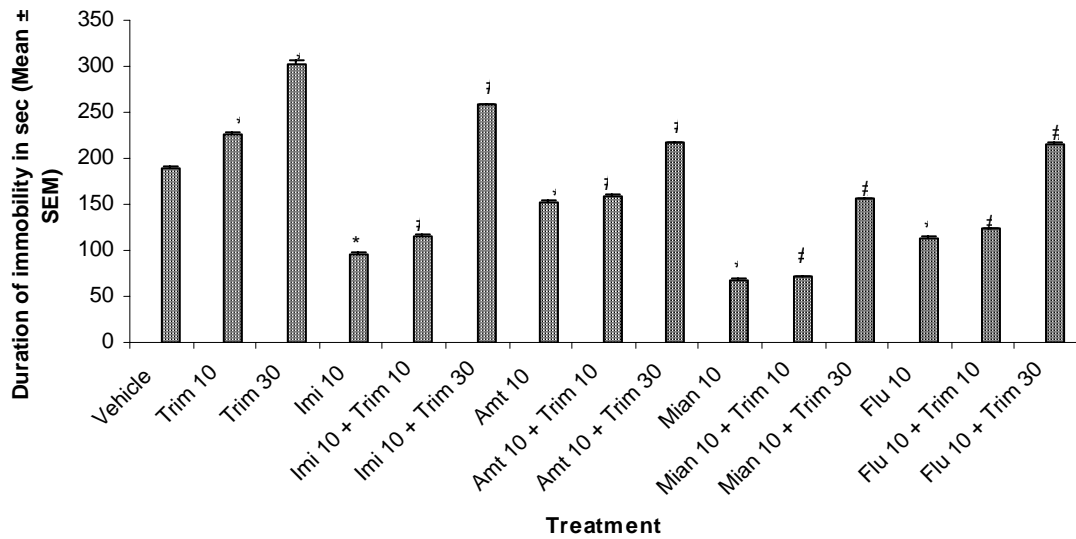


Fig. 3. Effect of trimyristin and antidepressants on duration of immobility in the tail suspension test $n = 5$, $F = 87.65$, $P < 0.0001$ (One-way ANOVA). * $P < 0.05$ compared to the vehicle treated group (Dunnett's test). # $P < 0.05$ compared to trimyristin treated groups (Dunnett's test).