

RESEARCH ARTICLE

ANXIOLYTIC EFFECT OF MYRISTICA FRAGRANS

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**Abstract:**

The use of *Myristica fragrans* as an anxiolytic agent has been mentioned in the ayurveda but still no study has been reported. The present study was evaluated for its anxiolytic activity using Open field model. In the open field test, the parameters measured were ambulation, rearing, grooming, and fecal pellets. In the open field test the *Myristica fragrans* at doses of 25mg/kg and 50mg/kg significantly increased the number of ambulations and rearing. The *Myristica fragrans* at doses 25mg/kg and 50mg/kg significantly decreased the number of grooming and fecal pellets. *Myristica fragrans* showed anxiolytic activity in dose dependant manner. *Myristica fragrans* at doses of 25mg/kg and 50mg/kg possess anxiolytic activity in the model tested and was found that higher dose (50mg/kg) was more significant than lower dose (25mg/kg). More investigations are necessary to prove the anxiolytic activity of *Myristica fragrans* by other models. Further the molecule responsible for the anxiolytic activity can be separated and identify the mechanism of action.

**Key words:** *Myristica fragrans*; Open field; ambulation, rearing, grooming, fecal pellets.

**INTRODUCTION**

Anxiety is a normal response to stress, a feeling of apprehension or fear, combined with the symptoms of increased sympathetic activity. A clinical problem may arise if anxiety becomes persistent that interferes with everyday performance. Clinical symptoms of anxiety include panic disorder, agoraphobia and other phobias and generalized anxiety [1]. The prevalence of such syndromes in the general population is about 10-20% and there is high rate of co

morbidity with depressive disorders [2]. The overall female to male ratio is 2:1. Although the maximum prevalence of generalized anxiety and agoraphobia-panic is in 50-64 age groups; the age of onset of most of anxiety disorders is in the young adulthood (twenties and thirties) [3]. Current pharmacotherapy of anxiety revolves around the use of synthetic molecules. However the drugs in current use are associated with the side effects such as drowsiness, impaired

motor activity, and abusive tendencies. The search for new drugs with better pharmacological profiles therefore continues. The alternative systems of medicines play an important role in the development of safe and effective drugs coming into the market.

Nutmeg (In Kannada-Jakayi/Jatiphala) consists of dried kernels of seeds of *Myristica fragrans* (Myristicaceae), an evergreen tree about 10-20 m high, indigenous to Moluccas Islands. The plant is now widely cultivated not only in Indonesia and Malaysia but also in Ceylon and West Indies (Grenada). In India it is cultivated in Kerala and Tamil Nadu. Current world demand for Nutmeg stands at about 10,000 tons per annum of which about 75% originates from Indonesia and 15 % from Grenada [4]. Nutmeg contains about 10% essential oil, which is mostly composed of terpene hydrocarbons (sabinene and pinenes; furthermore camphene, *pcymene*, phellandrene, terpinene, limonene, myrcene, together 60 to 80%), terpene derivatives (linalool, geraniol, terpineol, together 5 to 15%) and phenylpropanoids (myristicin, elemicin, safrol, eugenol and eugenol derivatives, together 15 to 20%). Of the latter group, myristicin (methoxy-safrole, typically 4%) is responsible for the hallucinogenic effect of nutmeg. Both nutmeg and mace contain about 2% of lignanes (diarylpropanoids), which are nonvolatile dimers of phenylpropanoid constituents of the essential oil, e.g; dehydrodiisoeugenol.

In traditional medicine, nutmeg and nutmeg oil were used for illnesses related to the nervous and digestive systems. Myristicin and elemicin are believed to be the chemical constituents responsible for the subtle hallucinogenic properties of nutmeg oil. Other known chemical ingredients of the oil

are  $\alpha$ -pinene, sabinene,  $\gamma$ -terpinene and safrole. Externally, the oil is used for rheumatic pain and, like clove oil, can be applied as an emergency treatment to dull toothache. Drops are put on a cotton swab, and applied to the gums around an aching tooth until dental treatment can be obtained. In France, it is given in drop doses in honey for digestive upsets and used for bad breath. Drops are put on a sugar lump or in a teaspoon of honey for nausea, gastroenteritis, chronic diarrhea, and indigestion. Alternatively massage oil can be created by diluting the essential oil in almond oil. This is sometimes for muscular pains associated with rheumatism or overexertion. It is also combined with thyme or rosemary essential oils. It should be noted that these are folk remedies. Nutmeg when ingested can be fatal and when applied to the skin it can be an irritant.

The ethanolic extract of *Myristica fragrans* was reported in rabbits. Rabbits treated with ethanolic extract of nutmeg showed significantly lower levels of total cholesterol, LDL-Cholesterol and triglycerides. Levels of HDL-Cholesterol were not significantly different from that of the control animals [5, 6]. Methanol extract of *Myristica fragrans* was reported to possess antiinflammatory activity. This illustrates that *Myristica fragrans* inhibits Nitric Oxide production and which is a mediator of inflammation [7]. It has been reported that the lignans from *Myristica* seeds possesses antioxidant activity against low density lipoprotein [8]. *Myristica fragrans* seeds have also been reported to reduce the volume and acidity of carbachol induced gastric secretion in fasting rabbits. These results indicated that the extract can be used effectively in peptic ulcers [9]. Pharmacological effects of Ligroin extract of *Myristica fragrans* was evaluated in chicks. The extract caused significant

increase in the duration of light and deep sleep. It was suggested that trimyristin present in the extract alters intensity and duration sleep induced by *Myristica fragrans* [10]. The use of *Myristica fragrans* as an anti-anxiety agent has been mentioned in the Ayurveda but still no study has been reported [11]. Ayurvedic literature insists the use of aqueous extracts in the formulations; no experimental evidence is reported regarding the testing of such aqueous extracts. Hence aqueous extract of *Myristica fragrans* is being tested for the anti-anxiety activity using Open field test model.

## MATERIALS AND METHODS

### Plant Extract, Drugs and other chemicals

The aqueous extract of *Myristica fragrans* was provided by Natsyn Catalysts, Tamil Nadu. The strength of the extract was 1: 10 meaning 1mg aqueous powder extract is equivalent to 10 mg of crude drug. According to the ayurvedic literature the dose for anti-anxiety activity is 0.5-1 g of crude drug/ human/day.<sup>4</sup> Based on this, two doses of aqueous extract of *Myristica fragrans* (25mg/kg and 50mg/kg) were selected for the study. The marketed preparation of the Diazepam injection (10mg) Calmpose, (Ranbaxy Pvt Ltd) was used as standard reference drug. 1 ml of the injection was dissolved in the sterile water for injection and was used for the experiment. All other chemicals and reagents used in this study were of analytical grade.

### Experimental Animals

Experimental study was carried out using Albino mice weighing between 18-35g. The animals were procured from Drug Testing Laboratory, Bangalore. All the animals were maintained under standard laboratory

conditions i.e. temperature of  $20 \pm 20^{\circ}\text{C}$ ; relative humidity 45-55 % and a 12 hour light/ dark cycle. The animals were fed standard mice pellet *ad libitum* under hygienic conditions. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The approval from the Institutional Animal Ethical Committee (Proposal No.IAEC/NCP/05/09) was taken prior to the study.

### Open Field Test Model [12]

This test utilizes behavioral changes in the rodents exposed to novel environment and has been used to detect anti-anxiety activity under identical situations. Since the exposure to a novel environment is associated with emotionality, an anxious animal is one, which shows reduced normal behaviors like rearing and grooming, concomitant with augmented automatic activity resulting in increased defecation agents and attenuated by anxiolytics. The open field test is simple test, sensitive and reproducible and appears to be similarly influenced by different classes of anxiogenic and anxiolytics agents. The test drug treatment was carried for 7 days and standard drug was given 2 h prior to the experiment as per the animal data contained in Table:1.

### Treatment

The animals were acclimatized for a week after bringing to the laboratory with everyday handling. The animals were divided into four groups with ten animals in each group as follows:

- Group I: vehicle control
- Group II: Diazepam 2mg/kg p.o

- Group III: *Myristica fragrans* 25mg/kg p.o
- Group IV: *Myristica fragrans* 50mg/kg p.o

No of days of drug treatment: 7 days

The open field is made up of plywood (40X40cm) with high walls, of same dimensions. The entire apparatus is colored with gray color sunmica, except for black lines that divide the floor into 16 squares. A bulb of 40 W was focused onto the field from a height of 100cm from the floor. The entire room, except for the open field was kept dark in the experiment.

(1). Two hours after the oral administration of the *Myristica fragrans* (25mg/kg and 50mg/kg) and diazepam 2mg/kg, the animal were placed individually at one corner of the apparatus and observed for 5 min. (2). The following behavioral aspects like: Ambulation (Number of squares crossed by the mouse), Rearing (Number of times the animal stood on the rear paws), (Self-grooming) Number of times the animal groomed the facial region and licks/washed/scratched various parts of the body and Defecation (number of fecal pellets) were observed. (3). At the end of each trial the apparatus was wiped clean with dettol solution in order to eliminate any olfactory clues which might modify the behavior of next animal. (4). The procedure was conducted preferably in a sound attenuated room, with observation made from adjacent room via web camera attached to the computer system.

### Statistical Analysis

All data were analyzed using one way ANOVA followed by Dunnett's *t* -test.

## RESULTS

The lower dose of *Myristica fragrans* (25mg/kg) showed significant increase ( $P<0.05$ ) in the number of ambulations when compared with vehicle control. Administration of Diazepam and higher dose of *Myristica fragrans* (50mg/kg) showed significant increase ( $P<0.001$ ) in the number of ambulations when compared with vehicle control.

The lower dose of *Myristica fragrans* (25mg/kg) significantly increased ( $P<0.05$ ) in the number of rearings when compared with vehicle control. Administration of Diazepam and higher dose of *Myristica fragrans* (50mg/kg) showed significant increase ( $P<0.001$ ) in the number of rearings when compared with vehicle control.

The administration of Diazepam and lower dose of *Myristica fragrans* (25mg/kg) showed significant decrease ( $P<0.01$ ) in the number of grooming when compared with vehicle control. The higher dose of *Myristica fragrans* (50mg/kg) also significantly decreased ( $P<0.001$ ) in the number of grooming when compared with vehicle control.

The administration of Diazepam and lower dose of *Myristica fragrans* (25mg/kg) showed significant decrease ( $P<0.01$ ) in the number of fecal pellets when compared with vehicle control. The higher dose of *Myristica fragrans* (50mg/kg) also significantly decreased ( $P<0.001$ ) the number of fecal pellets when compared with vehicle control.

**Table 1: Animal data used for the study in open field model**

Species	Mice
Strain	Albino
Age and sex	1-2 months, male
Body weight	25-35g
No of animals in each group	n=10
No.of groups	four
Water	<i>ad libitum</i>
Vehicle for herbal drugs	Sterile water for injection

**Table 2: Effect of aqueous extract of *Myristica fragrans* on behavioral aspects**

Effect of aqueous extract of <i>Myristica fragrans</i> on ambulations				
GROUPS	Control	Diazepam 2mg/kg b.w	<i>Myristica fragrans</i> 25mg/kg b.w	<i>Myristica fragrans</i> 50mg/kg b.w
MEAN±SEM	33± 3.72	92.67±11.79***	71.17± 5.54*	94.83±9.30***
Effect of aqueous extract of <i>Myristica fragrans</i> on rearing				
MEAN±SEM	4.66± 0.80	16.33±1.11***	12 ±2.74*	16±1.89***
Effect of aqueous extract of <i>Myristica fragrans</i> on grooming				
MEAN±SEM	10± 0.51	4.55±1.31**	4.83±1.22**	2.33±0.76***
Effect of aqueous extract of <i>Myristica fragrans</i> on fecal pellets				
MEAN±SEM	7± 0.577	3.16±0.79**	3.3±0.9**	2.50±0.34***

Data was analyzed using one way ANOVA and Dunnett's *t* test.

All the groups were compared with vehicle control, n=10

\*P<0.05, \*\*\*P<0.001

## DISCUSSION

The importance of demonstrating preclinical evidence of putative anti-anxiety activity cannot be minimised. Chronical anxiety includes generalised anxiety disorders, phobias and panic attacks and their treatments are not restricted to anxiolytics but include antidepressants as well. It is also not apparent that which type of anxiety has been tested in a given paradigm. However, the importance of these animal models of anxiety in evaluation of new anxiolytics can hardly be minimised. We have found an open field test for evaluating anti-anxiety activity. However, the final proof for putative anxiolytic effect will come from the clinical testing. The novel anxiolytics would never come to fore without rodents test in the initial stages. There is a reason to believe that novel chemical entities will emerge from the plant kingdom in our search for newer, safer and more effective anti-anxiety agents. This is the first study demonstrating the anti-anxiety activity of the aqueous extract of *Myristica fragrans* using open field test. Pretreatment for 7 days appeared to induce optimal effects, and this is the basis for the drug schedule used in the present investigation. On the contrary the repeated administration of the diazepam induces tolerance; hence, it was administered acutely using a single dose.

In Open Field Test, Ambulation is related to attempts to escape from the novel environment, and is an important anxiety related behaviour in open field testing, which is sensitive to the action of anxiolytic drugs such as benzodiazepines [12]. In this present study *Myristica fragrans* at doses of 50mg/kg and 25mg/kg significantly increased the number of ambulation in dose dependant manner indicating the anxiolytic activity. Rearing is an aspect of exploratory behaviour and generally decreases when animal is placed in the novel environment,

and may increase when anxiolytic drugs are given [12]. In the present study it was found that the test drug *Myristica fragrans* at doses of 25mg/kg and 50mg/kg significantly increased the number of rearings in dose dependant manner indicating anxiolytic activity as mentioned above. Grooming behaviour generally increases with fear or anxiety in rodents and is an index of behavioural adaptation to a stressful situation [13-15]. Anxiolytic drugs decrease grooming in the open field test and in present study groups receiving *Myristica fragrans* at doses of 25mg/kg and 50mg/kg significantly reduced the grooming in dose dependant manner. Defecation is also a good indicator of emotionality in animals, and research shows that high emotionality is related to an increase in defecation [13-15]. The increase in the defecation was found due to the heightened autonomic activity where the *Myristica fragrans* at doses of 25mg/kg and 50mg/kg reduced the number of fecal pellets in dose dependant manner.

In the open field test the animals express their anxiety and fear by decreasing ambulation and rearing. The *Myristica fragrans* at doses of 25mg/kg and 50mg/kg significantly increased the number of ambulations and rearing. The animals express their anxiety and fear by increasing grooming behaviour and defecation due to the heightened autonomic activity. The *Myristica fragrans* at doses of 25mg/kg and 50mg/kg significantly decreased the number of grooming and fecal pellets.

## CONCLUSION

*Myristica fragrans* at doses of 25mg/kg and 50mg/kg possess anxiolytic activity in the model tested and was found that higher dose (50mg/kg) was more significant than that of lower dose (25mg/kg) when compared to



vehicle control. More investigations are necessary to prove the anxiolytic activity of *Myristica fragrans* by other models. Further the molecule responsible for the anxiolytic activity can be separated and identify the mechanism of action.

## CONFLICT OF INTEREST

There is no conflict of interest associated with the authors of this paper.

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