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 
Kerela 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 *α*-pinene, sabinene, *γ*-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.4 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 ± 20C; 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) 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

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

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

<table>
<thead>
<tr>
<th>Effect of aqueous extract of <em>Myristica fragrans</em> on ambulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUPS</td>
</tr>
<tr>
<td>MEAN±SEM</td>
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Effect of aqueous extract of *Myristica fragrans* on rearing

| MEAN±SEM | 4.66± 0.80 | 16.33±1.11*** | 12 ±2.74* | 16±1.89*** |

Effect of aqueous extract of *Myristica fragrans* on grooming

| MEAN±SEM | 10± 0.51 | 4.55±1.31** | 4.83±1.22** | 2.33±0.76*** |

Effect of aqueous extract of *Myristica fragrans* 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 is 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 ambulations 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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