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Original Research Article

Screening of aqueous extract of *Myristica fragrans* seed for sedative and sleep enhancing property in experimental animals

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ABSTRACT

Background: *Myristica fragrans* houtt or nutmeg, a commonly used spice is found to have several medicinal properties.
Aim: The main aim of our study is to evaluate sedative and hypnotic activity of aqueous extract of *Myristica fragrans* houtt seeds in experimental animals.
Materials and Methods: This is a preclinical study involving male and female Wistar albino rats and Swiss albino mice. Sedative and hypnotic properties were assessed using hole board test, open field apparatus, rota rod apparatus and thiopental induced sleeping time. *Myristica fragrans* aqueous extract(MFAE) was the test drug which was given at the dose of 200mg/kg body weight. Diazepam was used as the standard drug. Preliminary phytochemical analysis of MFAE was carried out.
Statistical Analysis: Data was analyzed using one way ANOVA (Analysis of Variance) followed by Tukey Krammer Test. P value < 0.05 was considered as significant.
Results: Hole board test and open field test showed decrease in exploratory and locomotor activity and rota rod test showed decrease in time period of fall compared to normal and thiopental induced sleeping time showed that the test drug decreased the onset time of loss of righting reflex and prolonged the regaining time of righting reflex indicating hypnotic activity. Preliminary phytochemical screening showed the presence of alkaloids, glycosides, saponins, flavonoids, tannins and fixed oils.
Conclusion: Based on results we concluded that aqueous extract of *Myristica fragrans* seeds possess significant sedative hypnotic property.

1. Introduction

Sleep is an essential component for sound body and mind. Sleep is characterised by altered consciousness, decreased interaction with surroundings as well as decreased voluntary activities. An adult individual requires average 7-8 hours of sleep every day. Insomnia is a major sleep disorder where the individuals will find difficulty in falling asleep and stay asleep. Insomnia can be long term, short term or transient. Chronic insomnia can lead to depression and can decrease the quality of life.[1]

Hypnotic drug induces and maintains sleep which is comparable to normal arousable sleep. Sedative drug decreases the excitement and calms the person without inducing sleep, though can cause drowsiness. Currently available sedative hypnotics in the market are benzodiazepines, barbiturates and newer non benzodiazepine group of drugs. They have numerous side effects like tolerance, dependence, hypersensitivity etc.[2] Newer herbal drug having sedative hypnotic property might be favourable to mankind. This study which is on evaluation of sedative hypnotic property of aqueous extract of *Myristica fragrans* seeds might be useful in finding a new drug to treat insomnia, nervousness and to sedate patient before surgical procedure.

Myristica fragrans Houtt commonly known as nutmeg, is a spice which is generally used in Indian cuisine as a sweetener, aromatic and savouring agent. Nutmeg is the

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dried seed kernel of *Myristica fragrans* Houtt (Family Myristicaceae). Besides its common use as a kitchen spice, in alternative medicine nutmeg has been used as a stimulant, anti-diarrheal, carminative, stomachic, tonic, and as aphrodisiac.[3,4] Studies have demonstrated following pharmacological actions like analgesic,[5] antifungal,[6] antimicrobial,[7] anti-inflammatory[8] as well as hepatoprotective[9] activities. Folklore use mentions nutmeg paste mixed with honey is given to infants who cry at night for no evident reason, to induce sleep

The objective of the study is screening for sedative and sleep enhancing activity of aqueous extract of *Myristica fragrans* houtt seeds in experimental animals (rats & mice).

2. Materials and Methods

This study was conducted in the Ethno pharmacology laboratory, Department of Pharmacology, Yenepoya Medical College, Mangaluru, after obtaining the approval from the Institutional Animal Ethics Committee (IAEC).

Healthy adult male and female Wistar albino rats of 3-4 months of age weighing 150-200g and adult male and female Swiss albino mice weighing 20-25 grams were used for the study. They were housed in clean polypropylene cages, three rats and three mice in each cage, under standard housing conditions.

Myristica fragrans seeds were used for the study. The seeds were collected from the *Myristica fragrans* tree grown in Dakshina kannada district. It was authenticated by Dr. Krishna Kumar, Department of Applied Botany, Mangaluru University. The seeds were dried and powdered using mixer grinder.

The dried seed powder weighed around –250 g. It was wrapped in a muslin cloth and extracted using 1000ml of distilled water in Soxhlet apparatus[10,11] maintained at around 70^{o}C - 80^{o}C for period of 3 days. The extract was concentrated in the Rota- vapor at 60^{o}C and subsequently in the water bath for evaporation of solvent for a period of 2 days. Aromatic brownish extract was obtained which weighed 27.9g. Total yield obtained was 11.6% w/w

The acute toxicity study of aqueous extract of *Myristica fragrans* seeds(MFAE) was done according to Organisation for economic co-operation and development(OECD) guidelines 425 limit test[12] at the dose of 2000mg/kg.

The MFAE was tested for solubility in distilled water, since it was soluble, distilled water was used as vehicle. The dose of MFAE was fixed to 200mg/kg after performing pilot study with 100 mg/kg and 200mg/kg. Since 200 mg/kg was better than 100 mg/kg, 200mg/kg MFAE was used as test dose. The required amount of test drug was calculated according to the body weight of animals and was administered orally for ten days. On 10th day, screening tests were performed one hour after oral drug administration. Standard drug used was diazepam.

23 wistar albino rats and 54 swisss albino mice were used for the study. They were grouped into three different groups containing six animals each. Following screening tests were conducted.

2.1. Test for sedative effect[10,13]

2.1.1. Hole board test

Swiss albino mice weighing 20-30g were used. Hole board apparatus measures 40 x 40cm in size with 16 holes of 3cm diameter each are evenly distributed. The hole board is elevated so that the mouse poking its nose into the hole does not see the bottom. Nose poking is considered as an indication of curiosity/ exploratory behaviour and is measured. One hour after the administration of test compound, the animals were subjected to tests for 5 minutes. The average count of nose poking of treated animals were calculated and compared with control and standard group.(Figure 1)

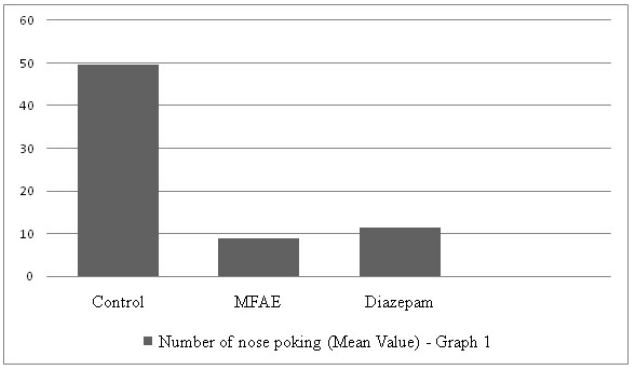

Fig. 1:

Fig. 2:

2.1.2. Open field test[10,13]

This test is to assess mobility or activity in the novel open field and emotionality of the animal. Wistar albino rats were used for the study. In an open field arena the motor activity of rats will be measured which is marked into 12 peripheral squares and 9 central squares. A rat when placed in the field explores the periphery normally. Activity or mobility in 5 minutes in the open field is quantified by keeping account of the number of squares crossed by the rats in the open field. Total number of squares crossed in different groups noted and compared. After each test arena was cleaned.(Figure 4)

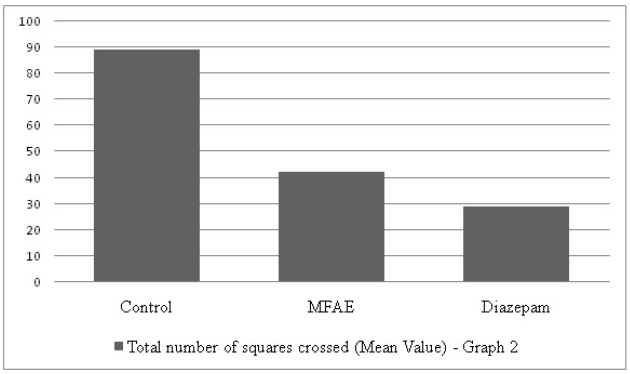

Fig. 3:

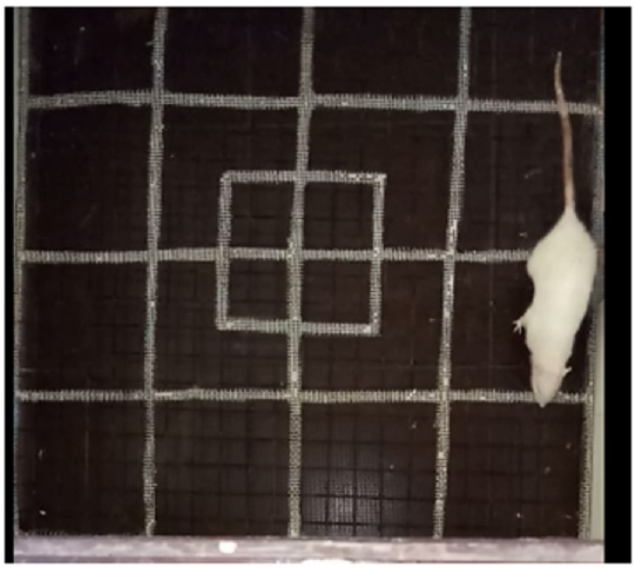

Fig. 4:

2.1.3. Test for motor coordination - rota rod test[10,13]

This test is performed using a horizontal rotation rod. Swiss albino mice undergo a pre test. Only those mice which can remain on the revolving rod for at least 60s were chosen for the test. 60min after oral administration of the test and standard drug the animals were placed on the rota rod for 1min. The number of animals falling from rota rod within the test period is calculated and it was compared with control.(Figure 6)

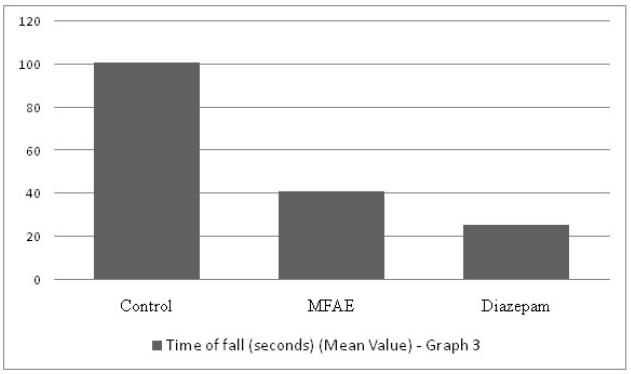

Fig. 5:

Fig. 6:

2.2. Test for sleep potentiating activity[10]

2.2.1. Potentiation of thiopental sodium induced sleeping time

Swiss albino mice weighing 20-30g were used. 60 min after oral dosing of test drug and standard drug, thiopental sodium administered at the dose of 20mg/kg intraperitoneally.[11] Animals were then placed on their back on thermostatically warmed pad. The duration of loss of righting reflex, starting from the time of administration of inducing agent, was measured until the animals regained their righting reflexes. (Figure 8)

2.3. Statistical analysis

Data was compiled and analyzed using the statistical package, GraphPad InStat software. Results are represented as Mean ± SEM (standard error of mean). Statistical significance between means was analysed using one way ANOVA followed by Tukey Kramer multiple comparison test. Values of $p < 0.05$ were considered as statistically significant and $p<0.01$ were considered statistically very significant and $p< 0.001$ were considered very highly significant.

3. Results

3.1. Sedative effect

3.1.1. Hole board test

MFAE treated mice showed decrease in the number of nose poking compared to control group. The p value of MFAE showed < 0.001 compared to control which suggests it is highly significant, but compared to standard drug diazepam it was not significant.(Table 1), (Figure 1)

Table 1: Results of hole board test

S. No	Group	Number of nose poking (Mean± standard deviation)
I	Control	49.66 ± 6.022
II	MFAE	8.833 ± 1.722*
III	Diazepam	11.5 ± 1.87

MFAE- *Myristica fragrans* aqueous extract 200mg/kg
One way ANOVA followed by Tukey Kramer test
*p value < 0.001- very highly significant compared to group I

3.1.2. Open field test

MFAE showed significant decrease in the mean movements of Wistar rats in the open field compared to control group**.** The p value of MFAE showed < 0.001 compared to control which suggests it is highly significant, but compared to standard drug diazepam it was not significant. (Table 2), (Figure 3)

Table 2: Results of open field test

S. No.	Group	Total number of squares crossed (Mean± standard deviation)
I	Control	89.16 ± 2.639
II	MFAE	42.16 ± 4.07*
III	Diazepam	29.16 ± 6.402

MFAE- *Myristica fragrans* aqueous extract 200mg/kg
One way ANOVA followed by Tukey Kramer test
*p value < 0.001- very highly significant compared to Group I

3.1.3. Rota rod test

Time of fall in MFAE treated group of mice was comparatively lesser than control group. The p value of MFAE showed < 0.001 compared to control which suggests it is highly significant. Compared to standard drug diazepam, p value was < 0.05 which was statistically significant.(Table 3), (Figure 5)

Table 3: Results of rota rod test

S. No.	Group	Time of fall (seconds) (Mean± standard deviation)
I	Control	100.833 ± 12.156
II	MFAE	41.16 ± 6.795*
III	Diazepam	25.83 ± 3.125

MFAE- *Myristica fragrans* aqueous extract 200mg/kg
One way ANOVA followed by Tukey Kramer test
*p value < 0.001- very highly significant compared to Group I
*p value < 0.05 – significant compared to Group III

3.1.4. Hypnotic effect-Potentiation of thiopental induced sleeping time

This showed that MFAE treated group of mice lost righting reflex earlier than the control and also gained back the righting reflex later than the control**.** It was found that P value of MFAE is < 0.001 compared to control. This signifies that it is statistically highly significant. (Table 4), (Figure 7)

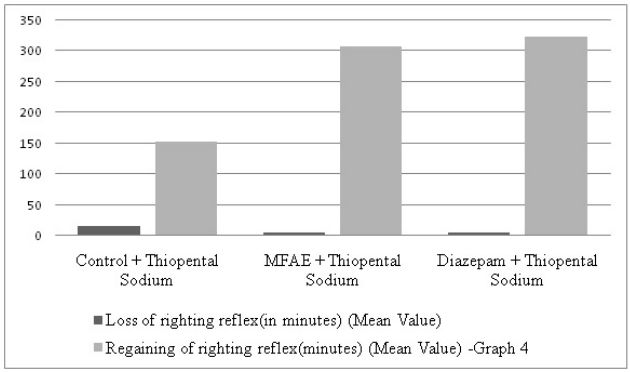

Fig. 7:

3.1.5. Phytochemical analysis of Myristica fragrans aqueous extract

Preliminary phytochemical analysis of MFAE showed presence of alkaloids, glycosides, tannins, saponins, fixed oil and flavonoids.

4. Discussion

Insomnia is one of the common complaints independently seen or associated with physical or mental disorders. Many hypothesis explains the pathophysiology of insomnia. The neurotransmitters involved in sleep are GABA, serotonin, acetylcholine and norepinephrine. Literature suggests that by enhancing GABAergic transmission, one can modulate $GABA_A$ receptors, which can result in sedation and

Table 4: Results of Potentiation of thiopental induced sleeping time

S. No.	Group	Loss of righting reflex(in minutes)	Regaining of righting reflex (minutes) (Mean± standard deviation)
I	Control+ thiopental sodium	16 ± 3.225	152.67 ± 5.007
II	MFAE + thiopental sodium	5.5 ± 1.517	307.66 ± 8.595*
III	Diazepam + thiopental sodium	5.66 ± 1.211	323.16 ± 16.822

MFAE- *Myristica fragrans* aqueous extract 200mg/kg
One way ANOVA followed by Tukey Kramer test
*p value < 0.001- very highly significant compared to Group I

Fig. 8:

hypnosis.[14,15]

The conventional sedative hypnotics like barbiturates and benzodiazepines act primarily at the GABA. They increase the GABA level which is an inhibitory neurotransmitter that lead to central nervous system depression. One of the main concerns is development of tolerance and dependence with these drugs.[14,15] So the plant based drug might have a better clinical efficacy and lesser side effect profile.

Hole board apparatus is used mainly to know whether the drug has got CNS stimulant or sedative property. Decrease nose poking in hole board suggest that there is some amount CNS depression and sedation.[10] MFAE showed significant decrease in nose poking compared to control which suggests that some component in seeds of *Myristica fragrans* aqueous extract possess sedative property.

The open field is one of the most popular procedure to test the locomotor activity and animal behaviour. Different versions are available, however a simple version where the total number of squares crossed in 5 minutes gives sedative or CNS depressant effect of the drug. It was found that MFAE produced significant decrease in movements compared to control. This shows that the extract has got sedative property.

Rota rod test is used to evaluate drugs which interfere with motor co-ordination.[10] The Swiss albino mice treated with MFAE lost their motor co-ordination earlier compared to control. This indicates that a component in the extract has got sedative property. The standard drug diazepam which is benzodiazipene also impairs motor co-ordination by acting on $GABA_A$ receptor. Since the MFAE action is similar to diazepam most probable mechanism of sedative activity is by activating GABA receptor and increasing the levels of GABA.

Potentiation of thiopental induced sleeping time is used to assess hypnotic activity of a drug.[10] MFAE is increasing the sleep duration and also there is decrease in time latency of loss of righting reflex it can be concluded that the extract is having GABA enhancing property which lead to potentiation of sleep.

Phytochemical analysis of MFAE showed presence of flavonoids, saponins, tannins and alkaloids. Flavonoids have demonstrated anxiolytic, sedative and anticonvulsant activities. Studies have shown that these effects are mediated by ionotropic GABA, in particular $GABA_A$ receptors. It is already known that chalcone a type of flavonoid is positive allosteric modulator of $GABA_A$ receptor has got hypnotic effect.[16,17] The possible mechanism can be proposed as the chalcone a flavonoid component present in MFAE positively modulates GABAA receptor thereby increasing the levels of GABA, leading to sedative and hypnotic activity.[17]

Limitation of the study: Our study was a preclinical study invoving screening methods to evaluate sedative and hypnotic activity. Further studies on neurotransmitter levels will confirm the above findings.

5. Conclusion

Preclinical evaluation of the aqueous extract of nut meg seeds were done for sedative and hypnotic activity in rats and mice. Phytochemical screening was also performed to know the components present in it. Based on above result it can be concluded from this study that MFAE (*Myristica fragrans* seed aqueous extract) 200mg/kg shows significant sedative and sleep enhancing activity. Further isolation of flavonoid component from the extract will be helpful in confirming the above findings.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare that there is no conflict of interest.

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