Original article

CNS Activity of Myristica fragrans Houtt. - An Experimental Study

Mishra A[1], Rahman SZ[2], Khan RA[3]

Abstract

Objective: To evaluatethe role of *Myristica fragrans Houtt*. for its analgesic, sedative and anxiogenic activity. ***Method:*** Charles Foster rats were administered orally the ethanolic extract (EEMF) and fractions of extract in ethyl acetate (EAMF), chloroform (CMF), and n-hexane (HMF). They were screened for sedative activity using Pentobarbitone sleep potentiation test, analgesic activity using Eddy's hot plate and Tail flick test; and anxiogenic or anxiolytic activity using Open field test. ***Result:*** In pentobarbitone induced sleep potentiation test, the ethanolic extract (EEMF) and ethyl acetate fraction (EAMF) showed significant decrease in latency of sleep and highly significant increase in duration of sleep. In Open field test, EEMF, EAMF and HMF showed highly significant decrease in locomotion parameters as shown by decrease in rearing, preening and ambulation. In Eddy's hot plate test and Tail flick test, highly significant increase in reaction time was seen in EEMF, EAMF, HMF groups. ***Conclusion:*** The observed results suggest that *Myristica fragrans* has analgesic, anxiogenic and sedative activity.

Keywords: *Myristica fragrans* Houtt.; Pentobarbitone; Open field behavior; Analgesic; Anxiogenesis

DOI: http://dx.doi.org/10.3329/bjms.v17i1.35289
Bangladesh Journal of Medical Science Vol. 17 No. 01 January'18. Page : 98-106

Introduction:

Since long time, aromatic spices have been added as ingredients to improve the taste and flavor of food. These aromatic spices are also commonly used in phytotherapy and mostly related to various activities of their constituents. Nutmeg (*Myristica fragrans Houtt.)* is the seed kernel of the fruit while, mace is the lacy covering (aril) on the kernel[1]. Nutmeg has been studied to having antihelminthic, hepatoprotective, anti-oxidant[2], aphrodisiac, and insecticidal properties and also used for treatment of rheumatism, diarrhea, asthma, atherosclerosis[3].

Sleep is a physiological process that is needed for recuperation. Sleep disorder has a relatively high prevalence and is a growing public health problem. On an average, more than 27% of population suffers from sleeping disorders with problem in initiating or maintaining sleep. It is estimated,that by the mid 21st century, approximately, 3-10% of all people will be frequent users of sleep medications[4,5]. Chronic sleep disorder leads to poor memorizing, emotional disturbances, slower reactions and changes in the immune response[6]. Benzodiazepines are the most commonly used agents for sleep disorders. They are responsible for unpleasant adverse effects which include drug dependence, tolerance, rebound insomnia, amnesia, psychomotor impairment and potentiating other central depressant drugs[7]. For these reasons, newer hypnotic agents with better safety profile are needed.

Anxiety is a pathological state of aggravated fear associated with motor tension, sympathetic over-activity,vigilance syndrome and apprehension leading to impairment of memory, intelligence and psychological function[8].The anxiety disorder consists of spectrum of panic disorder, generalized anxiety disorder, phobias, separation anxiety disorder and post traumatic stress disorder[9]. One-

1. Ankita Mishra
2. Syed Ziaur Rahman
3. Rahat Ali Khan

Department of Pharmacology, Jawaharlal NehruMedical College, Aligarh Muslim University, Aligarh, India

Correspondence to: Syed Ziaur Rahman, Professor, Department of Pharmacology & Deputy Medical Superintendent, Jawaharlal Nehru Medical College Hospital, Aligarh Muslim University, Aligarh 202002, India. Email: rahmansz@yahoo.com

eighth of the population in the world suffers from anxiety disorder[10]. It is more common in the female population as compared to males[11]. Recent trends have shown that one in every four Indians is affected by anxiety disorders[12].

Pain is one of the most common nonspecific manifestations of many diseases. Although non-steroidal anti-inflammatory drugs (NSAIDs) and opiates forms the basis of treatment for most of the painful conditions, but they are associated with several adverse reactions like renal damage, respiratory depression, gastrointestinal disturbances, and possible dependence[13, 14]. So there has been an increasing trend these days to find analgesics with lesser side effect profile from natural sources and medicinal plants.

The present study evaluates the analgesic, sleep-prolonging and anxiogenic effect of *Myristica fragrans*.

Material and Methods:

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC), Jawaharlal Nehru Medical College, A.M.U., Aligarh (Ref. no. 8662/ CAH dated 21.03.2016.). All animal experiments were carried out as per the rules and regulations framed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Materials:

A. *Drugs & chemicals used*

1. Morphine sulphate (Inj. Morphitroy15mg (1ml), Troikaa Pharmaceuticals Ltd., India).
2. Pentobarbitone sodium (Sigma Aldrich, USA).
3. Pentazocine (Inj. Fortwin30mg (1ml), Sun Pharmaceuticals Ltd., India).

B. *Plant Material*

Seeds of *Myristica fragrans* were obtained from the local market of Aligarh. These were identified and authenticated by Raw Material Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. A sample specimen of plant material was deposited in the NISCAIR bearing voucher numbers "NISCAIR/RHMD/ Consult/2015/2898/91" for *Myristica fragrans.*

C. *Preparation of extract and fractions*

Seeds of *Myristica fragrans* were crushed to a powder and extracted with 99.5% ethanol in a Soxhlet apparatus.Ethanolic extract of *Myristica fragrans* [EEMF] -dark brown, pleasant aroma, semi solid mass with extractive value of 23.63 %

A portion of ethanolic extract [EEMF] was sequentially fractionated with n-hexane, chloroform and ethyl acetate. The Extractive value was-Ethyl acetate fraction [EAMF] (17.91%), Chloroform fraction [CMF] (21.92%), n-Hexane fraction [HMF] (26.15%)[15].

D. *Laboratory Animal*

Charles Foster albino rats of either sex, weighing 100-150 g were used in the study. The animals were procured from the Central Animal House, J.N.M.C.H., A.M.U., Aligarh They were housed in polypropylene cages bedded with husk in the Pharmacology Section of Central Animal House and provided with standard pellet diet (Ashirwad Industries, Chandigarh) and water ad libitum. The animal room was well-ventilated and maintained under standard environmental conditions throughout the experiment (temperature 18-29ºC, humidity 30-70%, 12 hour light/dark cycle). They were acclimatized to the laboratory condition for 1 week prior to experimental use.

Methods:

Acute toxicity study**:**

Since earlier study from the same lab reported the safety of ethanolic extract of *Myristica fragrans* seeds[16, 17] thus, toxicity study was done for three fractions of *Myristica fragrans* seeds on healthy adult female rats (100-150g)as per Organization for Economic Cooperation and Development (OECD) Guidelines 425.

Each fraction of *Myristica fragrans* (MF) was administered per oral to five animals at a dose of 2gm/kg. All animals survived at the end of 14 days of observation. Accordingly, the LD_{50} of each fraction is greater than 2000 mg/kg.

Pentobarbitone induced hypnosis potentiation test:

The animals were given (per oral) a single dose of extract and fractions. After 1 hr, pentobarbitone (30 mg/Kg, IP) was injected to induce sleep. The ratswere considered asleep if they stayed immobile and lost their righting reflex when positioned on their back. The time interval between pentobarbitone injection and onset of sleep was recorded as sleep latency and the total sleeping duration (the time between the loss and the recovery of the righting reflex) were determined for each rat. The rat was considered as being awake if it could right itself (return to upright position). Various groups (n=6) were divided as follows (Table1).

Table 1: Experimental design (Pentobarbitone hypnosis potentiation test)

Groups	Medication
Group I (Pentobarbitone group)	Propylene glycol 0.3 ml/100g p.o. one hour before Pentobarbitone 30 mg/kg i.p.
Group II	EEMF 200mg/kg p.o.one hour before Pentobarbitone 30 mg/kg i.p.
Group III	EEMF 400mg/kg p.o. one hour before Pentobarbitone 30 mg/kg i.p.
Group IV	EAMF 200mg/kg p.o.one hour before Pentobarbitone 30 mg/kg i.p.
Group V	EAMF 400mg/kg p.o.one hour before Pentobarbitone 30 mg/kg i.p.
Group VI	CMF 200mg/kg p.o.one hour before Pentobarbitone 30 mg/kg i.p.
Group VII	CMF 400mg/kg p.o.one hour before Pentobarbitone 30 mg/kg i.p.
Group VIII	HMF 200mg/kg p.oone hour before Pentobarbitone 30 mg/kg i.p.
Group IX	HMF 400mg/kg p.oone hour before Pentobarbitone 30 mg/kg i.p.

EEMF-Ethanolic extract *Myristica fragrans*, EAMF-Ethyl acetate fraction of *Myristica fragrans*, CMF-Chloroform fraction of *Myristica fragrans*, HMF-n-Hexane fraction of *Myristica fragrans.*

Open Field Behaviour test:

The open field apparatus was made of plywood and consisted of sixteen 18 x 18 cm squares with a central square (18 cm x 18 cm) in the middle. The rats in each group were first trained for few days[18]. One hour after test drug administration, the rats were placed individually in the open-field arena three times after a gap of 3 minutes each and observed for a period of 3 minutes using modified methodology by Rahman SZ et al.[16]. The parameters recorded in the open field were Ambulation, Rearing, Preening, Defecation count. The Test was done in 9 groups of 6 animals each as shown in Table 2.

Table 2: Experimental design (Open field Behaviour test)

Groups	Medication
Group I	Normal control
Group II	EEMF 200mg/kg p.o
Group III	EEMF 400mg/kg p.o
Group IV	EAMF 200mg/kg p.o
Group V	EAMF 400mg/kg p.o
Group VI	CMF 200mg/kg p.o.
Group VII	CMF 400mg/kg p.o
Group VIII	HMF 200mg/kg p.o
Group IX	HMF 400mg/kg p.o

EEMF-Ethanolic extract of *Myristica fragrans*, EAMF-Ethyl acetate fraction of *Myristica fragrans*, CMF-Chloroform fraction of *Myristica fragrans*, HMF-n-Hexane fraction of *Myristica fragrans*

Analgesic activity by Eddy's Hot Plate method:

This is one of the most common and reliable method used for screening of centrally acting analgesic agents. The present study was performed by using the Eddy's Hot Plate Analgesiometer (Orchid Scientifics, India). The hot plate is an electrically heated aluminum plate with a temperature ranging between 55˚ to 56˚C[19].

Rats of either sex (150-200 g) were used. The response noted was licking/biting of both paws. Before starting experiment rats were similarly screened and those responding in <6 sec were chosen for the study.

The selected animals were placed on hot plate to record the response. The reaction time was measured at the interval of 30, 60, 90,120,150,180, 210 and 240 minutes after the administration of control and test drugs. Propylene glycol 0.3ml/100g p.o. served as control whereas Pentazocine 30 mg/kg i.p. was administered as standard drug. The cut-off time for response reaction was 30 seconds. The plate was wiped clean every time with saline if urination/ defecation is found. The test was done in 10 groups as shown in Table 3.

Table 3: Experimental design (Eddy's Hot Plate method)

Groups	Medication
Group I	Propylene glycol(0.3ml/100g) p.o
Group II	Pentazocine 30mg/kg i.p
Group III	EEMF 200mg/kg p.o
Group IV	EEMF 400mg/kg p.o
Group V	EAMF 200mg/kg p.o
Group VI	EAMF 400mg/kg p.o
Group VII	CMF 200mg/kg p.o
Group VIII	CMF 400mg/kg p.o
Group IX	HMF 200mg/kg p.o
Group X	HMF 400mg/kg p.o

EEMF-Ethanolic extract *Myristica fragrans*, EAMF-Ethyl acetate, CMF-Chloroform,
HMF-n-Hexane fraction, p.o- per oral, i.p.-intraperitoneal; n=6.

Analgesic activity by Rat Tail flick Test:

The method is based upon the reaction of rats to heat stimulus applied to their tail. It was performed by using the analgesiometer (Orchid Scientifics, India). Rats of either sex (150-200 gm) were placed in restraining holder so that the tail between the hole and tail tip or single point 3-5 cm from the tip of tail were directly kept over heated nichrome wire. The time taken by the rats to withdraw the tail was recorded[20]. Heat intensity was adjusted such that the average withdrawn latency is 3-6 sec and a maximum cut-off time of 15 sec adopted to prevent undue tissue damage.Tail flick latency was tested at 30 min interval for 4 hours. The test was done in 10 groups (n=6) as shown in Table 4.

Table 4: Experimental design (Analgesic activity by Rat Tail flick test)

Groups	Medication
Group I	Propylene glycol 0.3ml/100g p.o.
Group II	Pentazocin 30mg/kg p.o
Group III	EEMF 200mg/kg p.o
Group IV	EEMF 400mg/kg p.o
Group V	EAMF 200mg/kg p.o
Group VI	EAMF 400mg/kg p.o
Group VII	CMF 200mg/kg p.o
Group VIII	CMF 400mg/kg p.o
Group IX	HMF 200mg/kg p.o
Group X	HMF 400mg/kg p.o

Statistical analysis:

All the values were expressed as Mean ± SEM. Statistical significance was calculated by one way ANOVA followed by post hoc Dunnett's multiple comparison test (software used- SPSS) Version 23. $P < 0.05$ was considered to be statistically significant.

Results:

Pentobarbitone induced hypnosis potentiation test:

Intraperitoneal administration of Pentobarbitone sodium at dose of 30 mg/kg body weight produced loss of righting reflex (Table 5) with mean latency and duration of 3.72 min and 153 min, respectively. In the test groups, latency and duration were maximum in the ethanolic extract group [EEMF]. Pretreatment with ethanolic extract of *M.f* at dose of 200 and 400 mg/kg body weight significantly potentiated loss of righting reflex with mean latency as 2.77 min and 2.58 min, respectively (Fig 1). Similarly, duration of loss of righting reflex was increased significantly to 204.1 min and 253.4 min, respectively for the same group (Fig 2). Ethyl acetate fraction of *Myristica fragrans* [EAMF] at higher dose of 400 mg significantly reduced the latency period (Fig 1). Duration of loss of righting reflex was significantly increased at both the doses of EAMF. The chloroform and n-hexane fractions of *M.f* were unable to produce any significant changes in the latency or duration of righting reflex.

Table 5: Effect of *Myristica fragrans* on Pentobarbitone Induced Hypnosis test

Group	Latency(Min)	Duration(Min)
Pentobarbitone 30mg/kg	3.72±.07	153.51±3.20
EEMF 200	2.77±0.17*	204.14±11.13***
EEMF 400	2.58±0.23**	253.±4.01***
EAMF 200	3.18±1.7	202.15±5.57***
EAMF 400	2.87±0.13*	237.52±6.52***
CMF 200	3.67±0.20	150.32±3.64
CMF 400	3.63±0.29	167.33±4.98
HMF 200	3.60±0.20	159.14±1.93
HMF 400	3.71±0.13	168.60±4.76

Values are expressed as Mean ± SEM, (n = 6) *P<0.05, **P<0.01, ***P<0.001 as compared to Pentobarbitone group.

Open Field Behaviour Test:

The rats in the test group were treated with the extract and fractions of *M.f.* at dose of 200 and 400 mg/kg p.o., while the rats in the control group were given the same volume of Propylene glycol in the same manner. One hour later, all the animals were tested thrice after a gap of three minutes each for three minutes

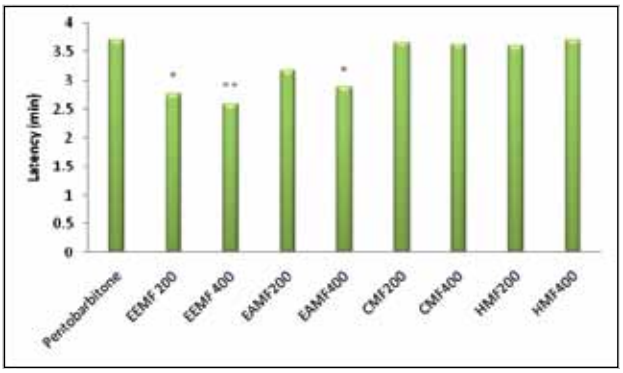

Fig 1: Effect of *Myristica fragran*s seed on Latency of loss of righting reflex on Pentobarbitone Induced Hypnosis test in rats.

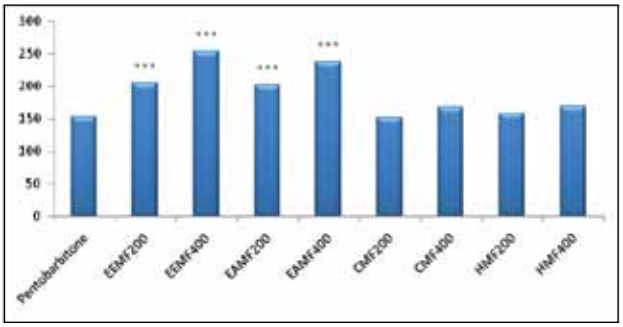

Fig 2: Effect of *Myristica fragrans* seed on Duration of loss of righting reflex on Pentobarbitone Induced Hypnosis test in rats

in the open field arena. As shown in Table 6, in the control group mean score of ambulation, rearing, preening and defecation count were 49.50, 17.67, 4.67 and 1.67 respectively (Table 6). Significant results were shown by the Ethanolic extract, Ethyl acetate fraction and n-Hexane fraction of *Myristica fragrans*. Ethanolic extract and n- hexane fraction of *Myristica fragrans* at dose of 400 mg/kg p.o. showed maximum and almost similar decrease in mean score

Table 6: Effect of *Myristica fragrans* on Open Field BehaviourTest

Group	Ambulation	Rearing	Preening	Defecation Count
Normal Control	49.50±2.26	17.67±2.59	4.67±0.82	1.67±0.51
EEMF 200	38.17±2.23***	16.88±4.86	2.73±1.03*	2.17±0.60
EEMF 400	27.83±2.99***	12.66±3.4*	2.23±0.52***	2.83±0.70
EAMF 200	44.50±1.38*	16.83±2.92	3.83±1.33	2.50±1.04
EAMF400	29.00±5.9***	14.33±2.58	4.00±1.41	2.67±1.03
CMF 200	48.17±3.38	17.16±2.79	3.00±1.41	2.16±0.75
CMF 400	48.00±2.53	16.50±2.35	3.16±1.17	2.25±0.75
HMF 200	42.17±2.17**	15.83±4.02	2.67±1.03*	2.64±1.63
HMF 400	26.33±3.61***	12.67±1.86*	2.07±1.21***	3.67±0.81**

Values are expressed as Mean ± SEM (n = 5) *P<0.05, **P<0.01, ***P<0.001 as compared to normal control. EEMF- Ethanolic extract of *Myristica fragrans.*

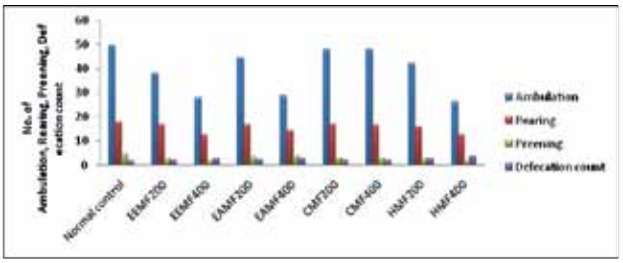

Fig 3: Effect of *Myristica fragrans* seed on Open Field Behaviour test in rats.

of Ambulation, Preening and Rearing. n-Hexane fraction (400mg) also showed significant increase in defecation count (Table 6). Ethyl acetate fraction of *Myristica fragrans* showed significant decrease in mean score of Ambulation only (Fig 3).

Eddy's hot plate Test:

The rats in the control group responded within cut-off time of 6 seconds in all time periods (Table7). The standard pentazocine group showed significant ($p<0.001$) increase in reaction time (seconds) - 5.49, 6.99, 8.15, 8.28, 6.89, 5.45 at interval (minutes) of 30, 60, 90, 120,150, 180 respectively. Ethanolic extract, HMF, EAMF showedsignificant increase in reaction time (seconds) as shown in table6; at interval (minutes) of 60, 90,120, 150 mins. The peak effect was seen at 120 mins.

Tail flick Test:

The rats in the control group responded within cut-off time of 6 seconds in all time periods (Table8). The standard pentazocine group showed significant ($p<0.001$) increase in reaction time (seconds) as5.21, 6.10, 9.10, 8.10, 7.54, 7.23, 5.50, at interval (minutes) of 30, 60, 90, 120,150, 180, 210 respectively. Ethanolic extract, HMF, EAMF showed highly significant increase in reaction time (seconds) at the interval (minutes) of 60, 90, 120,150 and 180 respectively. It was further noticed that mean response time was higher in the high dose group. The peak effect in both groups was seen at 90 mins.

Discussion:

Pentobarbitone enhances GABA receptor function or GABAergic neurotransmission in various brain regions[21] (Collingridge et al., 1984). The connection between GABA receptors activation and hypnotic response is still not known. Moreover, it is also not known

in which region the GABA receptors that mediate the hypnotic response are present. One of the hypothesis associated with sleep generation is that, the stimulation of GAB Aergic ventrolateral preoptic nucleus (VLPO) neurons during sleep inhibits nuclei involved in arousal such as the histaminergic neurons of tuberomammillary nucleus (TMN) or serotonergic neurons of the dorsal raphe nucleus (DRN)[22]. The effect of pentobarbitone is reported to be enhanced by additional sedative medicine. Serotonin has long been implicated in the regulation of sleep– wake states and it plays an important role in the initiation and maintenance of sleep[23, 24]. Although it is still not clear where and how serotonin exerts itseffect,as later it was found that serotonergic neurons play a role in inhibiting sleep[25].

Su- Ying et al., suggested that the potentiating effect of diltiazem on pentobarbital-induced sleep may be related to 5-HT_{1A} and 5-$HT_{2A/2C}$receptors, and DRN

Table 7: Effect of *Myristica fragrans* seed on reaction time in rats using Eddy's hot plate.

Group	0 min	30min	60min	90min	120min	150min	180min	210min	240min
Normal Control	3.69 ±.14	3.86±.14	3.89±.12	3.73±.22	3.75±.21	3.90±.19	3.69± .20	3.88±.19	3.85±.16
Positive Control	3.73 ±.20	5.49± .21***	6.99±.27***	8.15±.15***	8.28± .19***	6.89±.34***	5.85± .47***	4.12±.32	3.64±.20
EEMF 200	3.71±.05	3.90±.03	4.55±.06*	4.77±.07***	5.39±.12***	5.20±.07***	4.12±07	3.74±.12	3.71±.05
EEMF 400	3.84±.12	4.05±.10	4.86±.08***	5.24±.15***	5.92±.11***	5.28±.14***	4.19±.02	4.11±.07	3.78±.09
EAMF 200	3.67±.38	3.89±.04	4.49±.09*	4.69±.20***	5.01±.14***	4.52±.03*	4.02±.12	3.90±.05	3.91±.18
EAMF 400	3.90±.10	3.96±.13	4.76±.04***	4.90±.01***	5.12±.08***	4.92±.00***	3.92±.18	3.81±.10	3.72±.13
CMF200	3.85±.17	3.78±.20	3.82±.19	3.72±.20	3.90±.19	3.78±20	3.95±.09	3.80±.20	3.79±.10
CMF400	3.79±.20	4.00±.03	3.89±.07	3.84±.13	3.79±.16	3.81±.19	3.79±.20	3.84±.16	3.67±.21
HMF200	3.73±.15	3.90±.18	4.58±.09*	4.91±.04***	5.10.±05***	4.50±.09*	4.12±.15	3.80±.20	3.90±.06
HMF400	3.80±.19	4.10±.09	4.90±.05***	5.20±.10***	5.95±.05***	5.10±.10***	4.18±.02	3.78±.09	3.69±.21

Reaction Time: Mean±SEM (n= 6) sec. *P<0.05, **P<0.01, ***P<0.001 as compared to normal control.

Table 8: Effect of *Myristica fragrans* seed on reaction time in rats using Tail flick test.

Group	0 min	30min	60min	90min	120min	150min	180min	210min	240min
Normal Control	4.01±0.11	3.97±0.0	4.08±.08	4.04±.15	4.06±.01	4.03±.09	4.04±.14	4.01±.03	4.10±.00
Positive Control	3.97±0.08	5.21±.28***	6.10±.10***	9.10±.34***	8.10±.15***	7.54±.11***	7.23±.21***	5.50±.08***	4.13±.02
EEMF 200	4.02±0.14	4.09±.04	4.94±.11***	6.08±11***	6.01±.04***	5.13±.20***	5.13±.20***	4.10±.01	4.04±.02
EEMF 400	4.08±0.19	4.39±.05***	5.60±.07***	7.50±.15***	7.15±.11***	6.89±.15***	6.89±.15***	4.18±.02	4.02±.04
EAMF 200	4.01±0.12	4.05±.12	4.90±.05***	5.90±.13***	5.03±.18***	4.43±.1***	4.43±.11***	4.05±.10	4.07±.03
EAMF 400	4.06±0.10	4.25±.18***	5.10±.15***	6.05±.09***	5.91±.02***	4.98±.09***	4.98±.09***	4.08±.03	4.01±.10
CMF200	4.04±0.09	4.03±.02	4.10±.04	4.09±.08	4.04±.05	4.06±.10	4.06±.10	4.02±.08	4..10±.02
CMF400	4.04±0.02	4.05±.10	4.10±.10	4.02±.04	4.09±.01	4.03±.13	4.03±.10	4.00±.10	3.98±.11
HMF200	4.02±0.12	4.07±.11	4.89±.20***	5.98±.20***	5.43±.16***	4.81±.04***	4.81±.04***	3.98±.11	4.02±.09
HMF400	3.90±0.10	4.26±12***	5.40±.14***	6.99±.08***	6.64±.11***	5.40±.11***	5.40±11***	4.13±.02	4.04±.03

Reaction Time: Mean±SEM (n= 6) sec. *P<0.05, **P<0.01, ***P<0.001 as compared to normal control.

may be involved[26].

In our study, MF was found to potentiate pentobarbital-induced sleep. These observations points to the sedative effect of MF. The sedative effect may be due to the presence of various compounds in Myristica fragrans. Earlier study suggested that Myristica fragrans might be increasing the serotonergic activity in brain[27].

Another study by Sherry CJ suggested that the presence oftrimyristin present in *M.f* alters intensity and duration of sleep[28]. Another constituent of Myristica fragrans, myristicin is a safrole derivative with presence of methoxy group at carbon 4. The methoxy group in myristicin grants a strong sedative effect to nutmeg[29]. Moreover, it has been reported that myristicin is metabolized in the body, and its metabolite is a sedative, 3-methoxy-4,5-methylenedioxyamphetamine (MMDA)[30,31]. All these findings give strength to our study.

In the open-field test, we noticed a decrease in ambulation, rearing and grooming behavior in the HMF, EAMF and EEMF groups. These findings suggest that *M. fragrans* possess anxiogenic activity and are consistent with previous study by Sonavane et al., who also noted decrease in locomotion and rearing by both Hexane extract of *Myristica fragrans* and xrimyristin, thus possessing anxiogenic activity, whereas the anxiolytic agents increased both rearing as well as locomotion[32]. Decreased locomotion is an indicator of diminished dopaminergic transmission, which may be due to the rise in 5-HT level caused by anxiogenic agents[33]. Leiter et al. also suggested that myristicin does not reduce anxiety by GABA receptor modulation but may promote anxiogenesis. They concluded that myristicin antagonize the anxiolytic effects of midazolam, increase anxiety, and affect motor movements[34]. Muchtaridi et al. reported that myristicin, 4-terpineole and safrole were associated with inhibition of locomotor activity in mice. They suggested that this inhibition by nutmeg seed essential oil is due to the direct pharmacological action of one or more of its constituents[35]. More investigations are needed to identify the anxiogenic principles of nutmeg and to know their mechanism of anxiogenesis as it can act as an experimental tool in the screening of anxiolytic agents.

In our study EEMF, EAMF and HMF showed significant increase in reaction time in both hot plate and tail flick test, suggesting towards the analgesic activity of *Myristica fragrans* Human and animal data in previous studies, with pure nutmeg compounds also noted CNS depressant activity[36,37,38]. Earlier researcher, Joyce OO et al., (2012) concluded that analgesic effect of ethanolic extract of *Myristica fragrans* is both peripherally and centrally significant[39]. Similarly, Olajide et al., 1999 reported that oral administration of the *Myristica fragrans* possessed a potent analgesic effect against acetic acid-induced writhing in mice[40].Lopes et al., 2009 reported that both responses of hot plate test (paw licking and jumping) integrate at supraspinal structures with the C and Aδ type I and II sensitive fibers participating in this model[41].

Our result obtained from present study showed significant analgesic effect in both hot plate and tail flick tests suggesting enrichment in components (primarily non-lipid and/or aromatic compounds) that might activate a spinally-mediated analgesic pathway. Further lack of various constituents in chloroform fraction showed no statistically significant effects. Further mechanistic studies are needed to better understand the analgesic action of nutmeg.

Conclusion:

In our study, *Myristica fragrans* has significantly potentiated the pentobarbitone induced sleep, it has also significantly increased the latency of reaction in Hot plate and Tail flick test and finally in the Open field arena it decreased the locomotor activity significantly. Hence, in conclusion we can suggest that Myristica fragrans has a complex CNS activity and acts as a sedative has analgesic and anxiogenic activity. These activities can further be explored to find a new plant based therapy helpful in sleep disorder and in painful conditions. Its anxiogenic activity can be used to screen anxiolytic agents in experimental model.

References:

1. Nadkarni K. Indian Materia. 3rd ed. Bombay: Bombay Popular Prakashan; 1988. MyristicaFragrans.
2. Mila J, Olivera P, Mladen M. Chemical Composition and Antioxidant Effect of Free Volatile Aglycones from Nutmeg (*Myristicafragrans*Houtt.) Compared to Its Essential Oil. *CroaticaChemicaActa*. 2006; 79(2): 209-214.
3. Burkill IH. A dictionary of the economic products of Malay Peninsula. Kuala Lumpur: Ministry of Agriculture, Malaysia.1996; 1547-1556.
4. Roth T. Prevalence, associated risks, and treatment patterns of insomnia. J. Clin. Psychiatry. 2005; 66:10–13.
5. Weyerer S, Dilling H. Prevalence and treatment of insomnia in the community: results from the Upper Bavarian Field Study. Sleep. 1991; 14: 392-398.
6. Orzel-Gryglewska J. Consequences of sleep deprivation. Int J Occup Med Environ Health. 2010; 23(1):95-114.
7. Uzun S, Kozumplik O, Jakovljievic M, Sedic B. Side effects of treatment with benzodiazepines. PsychiatrDanub. 2010; 22:90-93.
8. Lakshmi BV, Sudhakar M, Ramya RL. Anti-anxiety activity of *Moringaoleifera* assessed using different experimental anxiety models in mice. J Pharm Res 2014;8:343-348.
9. Baldwin DS, Anderson IM, Nutt DJ, Allgulander C, Bandelow B, den Boer JA, *et al*. Evidence-based pharmacological treatment of anxiety disorders, post-traumatic stress disorder and obsessive-compulsive disorder: A revision of the 2005 guidelines from the British Association for Psychopharmacology. J ClinPsychopharmacol 2014; 28:403-39.
10. Shankar KB, Anu EJ. Antianxiety effect of ethanolic extract of leaves of *Moringaoleifera* in Swiss albino mice. Arch Med Health Sci 2014; 2:5-7.
11. SathyanarayanaRao TS, Darshan MS, Tandon A, Raman R, Karthik KN, SaraswathiN, *et al.* Suttur study: An epidemiological study of psychiatric disorders in South Indian rural population. Indian J Psychiatry 2014; 56:238-245.
12. Megha S, Pallavi B. Anxiety depression stress scale (ADSS): A factor analytic study. Int J Indian Psychol 2016; 3:52-65.
13. Domaj MI, Glassco W, Aceto MD, Martin BR. Antinociceptive and pharmacological effects of metanicotina, a selective nicotine agonist. J. Pharmacol. Exp. Ther. 1999; 291:390-398.
14. Farshchi A, Ghiasi G, MalekKhatabi P, FarzaeiHossein NA. Antinociceptive Effect of Promethazine in Mice. Iran. J. Basic Med. Sci. 2009;12: 140-145.
15. Ghosh MN, Fundamentals of Experimental Pharmacology; 6th ed. Hilton & Company; 2015
16. Rahman SZ, Ali Khan R, Kumar A. Experimental study of the morphine deaddictionproperties of denudatum, Wall. BMC Compl Alt Med 2002; 2(1): 6.
17. *Z* Imran, RahmanSZ , Khan RA, P Mehtab. An Experimental Study of Ethanolic Extract of Myristicafragrans in Morphine Dependence. Bangladesh J of Medical Science 2016; 15(2): 224-229.
18. Kulkarni, S.K. &Dandiya, P.C. Psychopharmacologia. 1973; 33: 333.
19. Eddy NB**,** Leimbach D. Synthetic analgesics: II. Dithienylbutenyl and dithienylbutylamines. J PharmacolExp Ther.1953; 107: 385-393.
20. Davies O. L,Raventos J, Walpol A. L.A method for evaluation of analgesic activity using rats. Brit. J. Pharmacol 1946; 1: 255-264.
21. Collingridge GL, Gage PW, Robertson B. Inhibitory post-synaptic currents in rat hippocampal CA1 neurones. J Physiol. 1984; 356:551-64.
22. Saper CB, Chou TC, Scammell TE. The sleep switch: hypothalamic control of sleep and wakefulness. Trends Neurosci. 2001; 24:726-31.
23. Jouvet M. Sleep and serotonin: an unfinished story. Neuropsychopharmacology. 1999; 21:24S–7S.
24. Ursin R, Serotonin and sleep. Sleep Med Rev. 2004; 6:65-69.
25. Dugovic C. Role of serotonin in sleep mechanisms. Rev Neurol (Paris). 2001; 157(11 Pt 2):S16-9.
26. Su-Ying Cui, Xiang-Yu Cui, Juan Zhang, Zi-Jun Wang, Yu, Zhao-Fu Sheng, Xue-Qiong Zhang, Xiao-Lei Shi, Yong-He Zhang. Diltiazem potentiates pentobarbital-induced hypnosis via 5-HT1A and 5-HT2A/2C receptors: Role for dorsal raphe nucleus"; Pharmacol. Biochem. Behav. 2011; 99: 566-572.
27. Kahn RS, Van Prag HM, Wtzler S, Asnis GM, Barr G. Serotonin and anxiety revisited. BiolPsychiat. 1988; 23:189- 208.
28. Sherry CJ, Ray LE, Herron RE. The pharmacological effects of a ligroin extract of nutmeg (Myristicafragrans). J Ethnopharmacol. 1981; 6:61-66.
29. Foye W. Principles of Medicinal Chemistry. Vol. 2. LEA & FEBRIGER; Philadelphia, PA, USA. 1981;931.
30. Stein, U.; Greyer, H.; Hentschel, H. Nutmeg (myristicin) poisoning—report on a fatal case and a series of cases recorded by a poison information centre. Forensic Sci. Int. 2001, 118, 87-90.
31. Beyer, J.; Ehlers, D.; Maurer, H.H. Abuse of nutmeg (MyristicafragransHoutt.): Studies on the metabolism and the toxicologic detection of its ingredients elemicin, myristicin, and safrole in rat and human urine using gas chromatography/mass spectrometry. Ther. Drug Monit. 2006, 28, 568-575.
32. Sonavane GS, Sarveiya VP, Kasture VS, Kasture SB. Anxiogenic activity of Myristicafragrans seeds. PharmacolBiochemBehav. 2002 Jan-Feb; 71(1-2):239-44.

33. GH Jones, TD Hernandez, DA Kendall, CA Marsden, TW Robbins; Dopaminergic and serotonergic function following rearing in rats; *Pharmacol, BiochemBehav*, **43** 1992, 17-35.
34. Leiter E, Hitchcock G, Godwin S, Johnson M, Sedgwick W, Jones W, McCall S, Ceremuga TE. Evaluation of the anxiolytic properties of myristicin, a component of nutmeg, in the male Sprague-Dawley rat. *AANA J.* 2011; **79**(2):109-114.
35. Muchtaridi, Subarnas A, Apriyantono A, Mustarichie R. Identification of compounds in the essential oil of nutmeg seeds (MyristicafragransHoutt.) that inhibit locomotor activity in mice. *Int J Mol Sci.* 2010; **11**(11):4771-4781.
36. Truitt E, Callaway EBM, Krantz J. The pharmacology of myristicin: a contribution to the psychopharmacology of nutmeg. *Journal of Neuropsychiatry*. 1961;**2:** 205-221.
37. de Mello A, Carlini E, Dressler K, Green J, Kang S, Margolis S. Behavioral observations on compounds found in nutmeg. *Psychopharmacologia.* 1973;**31:** 349-363.
38. Sangalli BC, Chiang W. Toxicology of nutmeg abuse. *Clinical Toxicology.* 2000;**38**(6):671-678.
39. O O Joyce, N D Chinwe, P D Tabot, C C Bruno, JppKwaku. The analgesic effect of ethanolic extract of MyristicafragransHoutt (nutmeg) on mice.*Am J PharmTech Res* 2012;**2**: 265-70.
40. Olajide OA, Ajayi FF, Ekhelar AI, Awe SO, Makinde JM, Alada ARA. Biological effects of Myristicafragrans (nutmeg) extract. *Phytotherapy Research* 1999; **13**: 344-345.
41. Lopes L, Pereira S, Silva L, Figueiredo K, Moura B, Almeida F, Sousa F. Antinociceptive effect of topiramate in models of acute pain and diabetic neuropathy in rodents. *Life Sciences* 2009; **84**: 105-110.