Original article

An Experimental Study of Ethanolic Extract of *Myristica fragrans* in Morphine Dependence

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Abstract:

Objective: To evaluate the role of ethanolic extract of Myristica fragrans in morphine dependence. ***Methods***: Wistar albino rats were made moderate and severe grade morphine dependence by administering morphine sulphate in dose of 10 mg /kg (i.p.), twice daily for 4 days and by increasing doses of 10-100 mg/kg (i.p.), twice daily for 7 days, respectively. The signs of spontaneous abstinence syndrome were recorded 12 hours in both studies after the last dose of morphine for 30 minutes and quantified by 'counted' and 'checked' signs. Ethanolic extract of Myristica fragrans (EEMF) was administered p.o. in different regimen: (a) EEMF 200 mg/kg along with morphine twice daily for 4 days and 7 days in moderately and severely induced morphine dependence group, respectively. (b) EEMF 400 mg/kg (p.o.), single dose10 hours after the last dose of morphine in both moderately and severely induced morphine dependence rats. ***Result***: Oral administration of EEMF in both study groups caused significant reduction in the scores of counted and checked signs of morphine abstinence syndrome as compared to active morphine control group. The reduction was significantly more in regimen 'a' as compared to regimen 'b'. ***Conclusion***: Ethanolic extract of Myristica fragrans seed significantly reduced the mean scores of various'counted signs' and 'checked signs' of morphine withdrawal syndrome and might give a solution as a substitute therapy in morphine de-addiction.

Keywords: Myristica fragrans; De-addiction; morphine sulphate

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Introduction

Morphine addiction is worst affected socio-economic problem worldwide. It causes mild to severe dependence resulting into difficult withdrawal at the time of de-addiction. A number of therapeutic medicines have been described in indigenous system of medicine to overcome the addiction. *Myristica fragrans*(Nutmeg) belongs to the family Myristicaceae, is one of the important spices used in indigenous system of medicine in India. Its usefulness is reported in inflammation, cephalgia, helminthiasis, halitosis, dyspepsia, flatulence, nausea, vomiting, diarrhoea, dysentery, colic, asthma, catarrh, neuralgia, lumbago, stangury, amenorrhoea, menorrhagia, dysmenorrhoea, ulcers, liver and spleenic disorders, eye diseases, impotency, skin diseases, freckles, cracks in feet, insomnia, delirium tremens, hyperdypsia, cardiac disorders, fever and general debility[1].Its psychoactivity such as hallucinations, feelings of euphoria, unreality, and delusions is documented since the middle ages[2]. Under the cover of its

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nature as spice, it is also widely abused as a cheap substitute for morphine narcotic drugs, particularly in adolescents[3, 4], since the 12th century[5, 6]. Earlier, the pharmacological activity of another medicinal plant, *Delphinium denudatum*using the same morphine dependence model, was reported by our lab[7, 8]. However, the present study is done to evaluate the role of ethanolic extract of *Myristica fragrans* in morphine dependence.

Materials and Methods:

Plant Materials

Seeds of Myristica fragrans were obtained from the local market. These were identified and authenticated by Dr. (Mrs.) Sunita Garg, Chief Scientist, 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 number "NISCAIR/ RHMD/2013/2345-125-2".

Preparation of Extract

Myristica fragrans seeds were shade-dried. 100 g plant material was powdered with the help of an electrical grinder (REMI auto-mix blender, Vasai, India). For the preparation of ethanolic extracts, powdered seed were extracted with 300 ml of absolute alcohol with the help of Soxhlet apparatus. The extract was then filtered, collected and evaporated till it becomes dry at 40˚C on a water bath. The semisolid mass so obtained was weighed to calculate its yield in percentage. The yield of extract was 17.94%.

Animals Used

Wistar albino rats weighing 150-200 g of either sex were used in the study. The animals were procured from theInstitutional Central Animal House. 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 18-29□C, humidity 30-70%, 12 hour light/dark cycle). They were acclimatized to the laboratory condition for 1 week prior to experimental use. The study followed ARRIVE guidelines and was approved by the Institutional Animal Ethics Committee (IAEC).

Drugs

Morphine sulphate (Inj. Morphitroy*15, Troikaa Pharmaceuticals Ltd., India).

DE-ADDICTION STUDY

Moderately induced Morphine dependence

Morphinein dose of 10 mg /kg (i.p.), twice daily for 4 days caused development of moderate morphine dependence[9].Rats were divided into five groups of five animals each. Group I received Propylene glycol 0.3 ml/100g p.o. twice daily for 4 days and served as normal control (vehicle control) , Group II received ethanolic extract of *Myristica fragrans* (EEMF) 200 mg/kg p.o. twice daily for 4 days and served as drug control (positive control), Group III received Morphine Sulphate 10 mg/kg (i.p.), twice daily for 4 days and served as Morphine control (Active control), Group IV received morphine sulphate 10 mg/kg (i.p.), along withEEMF200 mg/ kg p.o. twice daily for 4 days, Group V received morphine sulphate 10 mg/kg (i.p.),twice daily for 4 days followed by single dose of EEMF400mg/kg p.o. 10 hours after thelast dose of morphine.

The morphine abstinence syndrome, on which the assessment of physical dependence of morphine was based, consisted of a variety of motor and vegetative signs. The signs of spontaneous abstinence syndrome were recorded 12 hours after the last dose of morphine for 30 minutes. Similar observations were also done in drug control and normal control groups. The 'counted signs' and 'checked signs' were multiplied with the respective 'weighing factors' for evaluation of the severity of abstinence syndromeusing a modified methods of Blasig J, et al. and Neil & Sparber [TableI] [10, 11].

Severely induced Morphine dependence:

Morphinein increasing doses 10-100 mg/ kg(i.p.),twice daily for seven days is reported to cause development of severe dependence (Table II). The withdrawal signs were observed 12 hours after the last dose of morphineinjection for 30 minutes in all groups[4].The 'counted signs' and 'checked signs' were multiplied with the respective 'weighing factors' for evaluation of the severity of abstinence syndrome as done for moderately induced physical dependence.

Statistical Analysis

Data is represented as Mean ± SEM and analysed using one-way ANOVA followed by Tukey multiple comparison tests. $P < 0.05$ was considered statistically significant.

Results

Effect of ethanolic extract of *Myristica fragrans* on parameters of abstinence syndrome in moderately induced morphine dependent rats

The mean score of counted and checked signs in

Table I: Signs observed in rats 12 hours after the last dose of morphine for 30 minutes.

Counted Signs	Weighing Factors	Checked Signs	Weighing Factors
Chewing	2	Scream on touch	1
Head Shake	2	Hostility on Handling	1
Exploring	1	Diarrhea	1
Digging	2	Eye Twitching	2
Yawning	2	Lacrimation	3
Teeth Chattering	2	Ptosis	2
Jumping	2	-	-
Wet Dog Shaking	2	-	-

Table II: Dose schedule of morphine in severely induced morphine dependence

Days	Time	Dose (mg/kg)
1st	12.00 Noon	10
2nd	12.00 Noon and 10.00 p.m.	10 and 20
3rd	12.00 Noon and 10.00 p.m	20 and 40
4th	12.00 Noon and 10.00 p.m	40 and 60
5th	12.00 Noon and 10.00 p.m	60 and 80
6th	12.00 Noon and 10.00 p.m	80and 100
7th	12.00 Noon and 10.00 p.m	100 and 100

morphine group (study group III) were significantly increased ($p < 0.001$) as compared to normal control (Table III).Administration of EEMF in dose of 200 mg/kg orally twice a day for 4 days did not produce any sign of physical dependence (study group II). There was no difference in the counted and checked signs in EEMF group from normal control group.

Administration of EEMF in dose of 200 mg/kg orally along with morphine 10 mg/kg(i.p.), twice daily for 4 days did not produce significant mean score of withdrawal signs compared to morphine control group. In other words, the co-administration caused significant reduction in scores of counted and checked signs of morphine abstinence syndrome as compared to morphine control group observed 12 hours after the last dose of morphine (study group IV).

In group IV, mean score of various counted and checked signs of morphine abstinence syndrome were markedly reduced as compared to group V except headshakes. Comparative to group III, the mean score of counted and checked signs such as chewing, headshakes, yawning, digging, teeth chattering and scream on touch were significantly decreased. However, no significant change was observed in hostility on handling compared to morphine control group [Table III].

Mean score of counted signs such as chewing, headshakes, yawning, digging and teeth chattering in study group V were significantly decreased. Similarly, mean score of checked signs such as scream on touch was significant reduced. However, no significant change was observed in hostility on handling compared to morphine control group (Table III).

Effect of ethanolic extract of *Myristica fragrans*on parameters of abstinence behaviour in severely induced morphine dependent rats.

Apart from chewing, head shakes, yawning, digging, teeth chattering, scream on touch and hostility on handling, which were observed during moderate abstinence syndrome, wet dog shakes, jumping, eye twitching and lacrimation were also observed in severely induced morphine dependent rats.Mean score of counted and checked signs in morphine group (study group III) were significantly increased ($p < 0.001$) compared to normal control (Table IV). These mean scores are more than the moderately induced morphine control group.

Administration of EEMF in dose of 200 mg/kg orally twice a day for 7 days did not produce any sign of physical dependence (study group II). Likewise, there was no difference in the counted and checked signs in EEMFgroup from normal control group.Administration of EEMFin dose of 200 mg/kg orally along with morphine (in increasing doses 10-100 mg/kg) for seven days caused significant reduction in scores of counted and checked signs of morphine abstinence syndrome as compared to morphine control group observed 12 hours after the last dose of morphine (study group IV).

Mean score of various counted and checked signs was markedly reduced as compared to group V except headshakes and eye twitching. Comparative to group III, the mean score of counted and checked signs such as chewing, yawning, digging, teeth chattering, jumping, wet dog shake and scream on touch, hostility on handlingwere significantly decreased. However, no significant change was observed inheadshakes, eye twitching and lacrimation compared to morphine control (Table IV).

Mean score of counted signs such as chewing, headshakes, yawning, digging, teeth chattering, Jumping and Wet dog shake in study group V were significantly decreased. Similarly, mean score on checked sign of scream on touch, hostility on handling, eye twitching were significant reduced

Table III: Effect of ethanolic extract of Myristica fragrans on parameters of abstinence syndrome in moderately-induced morphine dependent rats.

Groups	Chewing	Head shake	Yawning	Digging	Teeth Chattering	Scream on touch	Hostility On hand
Group I	1.2± 0.49	0.0±0.0	1.2±0.8	2.0±0.8	0.0±0.0	0.0±0.0	0.0±0.0
Group II	0.8±0.4	0.0±.0.0	0.8±0.4	2.4±0.7	0.0±0.0	0.2±0.2	0.0±0.0
Group III	11.6±1.1***	8.8±1.0***	14.8±1.0***	7.60±1.6***	10.4±1.3***	3.6±0.4***	1.6±0.7*
Group IV	3.2±0.4***	3.2±0.4***	4.0±0.6***	2.4±0.4**	4.0±0.6***	1.4±0.2***	1.4±0.2
Group V	4.4±0.7***	2.8±0.4***	4.8±0.4***	3.6±0.4*	5.2±0.4***	1.6±0.2***	1.6±0.4

Values are expressed as Mean ± SEM (n = 5) *P<0.05, **P<0.01, ***P<0.001. Comparisons between: Group III vs. Group I., and Group IV, Group V vs. Group III. Group I (Normal control), Group II (EEMF control), Group III (Morphine control), Group IV (EEMF200 test group), Group V (EEMF400 test group).

Table IV: Effect of ethanolic extract of Myristica fragrans on parameters of abstinence behaviour in severely morphine dependent rats.

Parameters	Group I	Group II	Group III	Group IV	Group V
Chewing	1.6±0.7	2.0±0.6	21.6±1.1***	10.4±0.7***	12.4±1.3***
Head shake	0.0±0.0	0.0±0.0	3.6±0.7***	2.8±0.4	1.6±0.7*
Yawning	0.8±0.4	1.6±0.7	17.6±0.7***	10.4±0.7***	12.0±0.6**
Digging	3.2±0.4	3.6±0.9	14.8±0.7***	9.6±0.7**	10.4±0.4**
Teeth chattering	0.0±0.0	0.0±0.0	19.2±1.0***	10.4±1.4***	11.6±0.7***
Scream on touch	0.0±0.0	0.2±0.2	4.0±0.3***	1.8±0.2***	2.0±0.3***
Hostility on handling	0.0±0.0	0.0± 0.0	4.8±0.3***	2.6±0.2***	2.6±0.2**
Wet dog shake	0.0±0.0	0.0±0.0	2.8±0.4***	0.0±0.0***	0.0±0.0***
Jumping	0.0±0.0	0.8±0.4	2.4±0.7***	0.0±.0.0***	0.0±0.0***
Eye twitching	0.0±0.0	0.0±0.0	4.4±0.7***	2.4±0.7	2.0±0.6*
Lacrimation	0.0±0.0	0.0±0.0	3.6±0.6***	1.8±0.7	2.4±0.6

Values are expressed as Mean ± SEM (n = 5) *P<0.05, **P<0.01, ***P<0.001. Comparisons between: Group III vs. Group I., And Group IV, Group V vs. Group III. Group I (Normal control), Group II (EEMF control), Group III (Morphine control), Group IV (EEMF200 test group), Group V (EEMF400 test group).

(P<0.001). However, no significant change was observed in lacrimation compared to morphine control (Table IV).

Discussion:

The study was conducted to evaluate the role of *Myristica fragrans* in suppressing the signs of withdrawal in morphine-dependent rats. In our set up, we could observe only seven counted signs, viz. chewing, head shakes, digging, yawning, teeth chattering, jumping and wet dog shakes and four checked signs, viz. scream on touch, hostility on handling, eye twitching and lacrimation out of all signs as mentioned in TableI.

Earlier studies also observed that some signs were more prominent with less dependence and disappear as the degree of dependence increases, while other signs appear[12]. Further, the intensity of tolerance and precipitated withdrawal in rats is a function of the dosage and the interval of administration of morphine[11].The results obtained in present study indicate that morphine sulphate in doses of 10 mg/kgfor 4 days caused development of moderate dependence.

EEMF caused reduction in the severity of abstinence syndrome both in groupsIV and V. The maximum reduction in withdrawal signswas observed in group IVwhere EEMF 200 mg/kg along with morphine was given. Mean score of counted and checked signs such as chewing($p < 0.001$), headshake ($p < 0.001$), yawning ($p < 0.001$), digging ($p < 0.01$), teeth chattering ($p < 0.001$) and scream on touch ($p < 0.001$), were significantly decreased. However, no significant change was observed in hostility on handling compared to morphine control

group.

Administration of morphine in increasing doses caused development of severe dependence. Reduction in the severity of the abstinence syndrome was observed in extractgroup which were treated with EEMF along with morphine in different dose regimens. In EEMF treated group maximum reduction in withdrawal signs was observed in group IVin which theEEMF was given in multiple doses (200mg/kg twice daily × 7 days) along with morphine. Mean score of counted and checked signs such as chewing ($p < 0.001$), yawning ($p < 0.001$), digging ($p < 0.01$),teeth chattering($p < 0.001$), jumping ($p < 0.001$), wet dog shake($p < 0.001$), and scream on touch ($p < 0.001$), hostility on handling($p < 0.001$)were significantly decreased. However, no significant change was observed in headshakes, eye twitching and lacrimation compared to morphine control, whereas the score of wet dog shakes and jumping fell to zero. It was observed that, groupVwhere the EEMFwas given in single dose (400 mg/kg twice daily × 7 days), the reduction in withdrawal signs was less as compared to group IVHowever, no significant change was observed in lacrimation compared to morphine control.

The present result showed that EEMF alone did not cause physical dependence as morphine group observed 12 hours after the last dose. However, the scores of various counted and checked signs in both treated test groups showed significant reduction in controlling the morphine withdrawal syndrome. Thus, EEMF has positive role in attenuating withdrawal signs of morphine abstinence syndrome. In addition, the inhibition of withdrawal signs in EEMF along with morphine treated group (group IV) showed cumulative nature of drug.Whereas inhibition in single dose of EEMF treated group (group V) showed rapid onset of action of drug.The higher protection rate in multiple doses of EEMF treated group (Group IV) as compare to single dose of EEMFtreated rats (Group V) appears to be due to higher dose effect, because the total amount of EEMF administered in 4 days and 7 days was 1600 and 2800 as compared to 400 in single dose treated group.

Beck T et al., 2001 reported that psychoactivity of *Myristica fragrans*is documented since the middle agessuch as hallucinations, feelings of euphoria, unreality, and delusions[2]. Further, Kelly BD et al., 2003 and Demetriades AK et al., 2005 evaluated that it is widely abused as a cheap substitute for morphine narcotic drugs, particularly in adolescents[3, 4].

Janssen and Lackman,1990 reported that *Myristica fragrans*volatile oil is comprised of a mixture ofterpenes and alkenylbenzene derivatives. Myristicin,safrole and elimicin constitute about 80% of thealkenylbenzene derivatives[13].El-Alfy A., 2009 reported that psychoactivity of *Myristica fragrans*has always been associated with the hypothesis of potential metabolic activation of nutmeg constituents to amphetamine-like compounds. They also reported that similar to classic hallucinogens, both myristicin and elemicin have been reported to elicit psychoactivity as well as binding to 5-HT receptors[14].

Demetriades AK et al., 2005 studied that the active substance *Myristica fragrans* is myristicin, it has a weak monoamine oxidase inhibitor action and with elemicin may be metabolised to an amphetamine-like compound with hallucinogenic effects similar to lysergic acid diethylamide[15]. Beyer J et al., 2006 postulated that psychotropic effects could be attributable to metabolic formation of amphetamine derivatives from the main nutmeg ingredients elemicin (EL), myristicin (MY), and safrole (SA) [16].

Muchtaridi et al., 2010 studied that Myristicin is a safrole derivative with methoxy group attached at carbon 4. They reported that myristicin undergoes metabolism in the body, and its metabolite [3-methoxy-4, 5 methylenedioxyamphetamine (MMDA)] is known to cause sedative[17].

In the light of the above findings, it has been noted that ethanolic extract of *Myristica fragrans* seed significantly reduces the mean scores of various‘counted signs‘ and ‚checked signs’ of morphine withdrawal syndrome. The presence of *Myristica fragrans* metabolites may be the explanation of decrease score of various counted and checked signs which manifested during morphine withdrawal. However, more studies are still needed to determine the effect of these compounds on central neurotransmitter release and activity.

Conclusion:

The ethanolic extract of Myristica fragrans significantly reduced the mean scores of various’counted signs’ and ‘checked signs’ observed as an inherent constituent of morphine withdrawal syndrome.The present drug is one of the important spices used in indigenous system of medicinewhich might give a solution as asubstitute therapy in morphine de-addiction.

References:

1. Phulsagar S,Dundi M, Bhagwat S, Girigaon Y. An Inside Review of MyristicaFragransHoutt. A Potential Medicinal Plant of India. 2014; 1 (9): 500-513.
2. Beck T, Marty H. Die Nervenkekse der Hildgard von Bingenkeine harmlose Nascherei. Schweiz Med Forum 2001;51/52:1287–1288.
3. Kelly BD, Clarke MM, Gavin BE, Lane AA, Larkin CC. Nutmeg and psychosis. Schizophrenia Research 2003; 60:95–96.
4. Demetriades AK, Wallman PD, McGuiness A, Gavalas MC. Low cost, high risk: accidental nutmeg intoxication. Emergency Medicinal Journal 2005;22:223–225.
5. Quin G, Fanning N, Plunkett P. Nutmeg intoxication. Letters, Notice, Book reviews 1998; 287–288.
6. Sangalli BC, Chiang W. Toxicology of nutmeg abuse. Clinical Toxicology 2000;38(6):671–678.
7. Rahman S Z, Khan RA & Kumar A, A Study of Delphinium denudatum Wall. in morphine dependence, In: SS Khan, Ed. Vistas in Ethnobotany. Vol 1. *Ind J Appl Pure Biol*;2000. P. 111-125.
8. Christina Chase, Study shows Delphinium denudatum aids in de-addiction of morphine. HerbClip, American Botanical Council. Available from: http://cms. herbalgram.org/herbclip/234/review43490.html/
9. Rahman S, Khan RA, Kumar A. Experimental study of the morphine deaddiction properties of *Delphiniumdenudatum*Wall. BMC Compl Alt Med, 2002; 2:6.
10. Blasig J, Herz A, Reinhold K, Zieglgansberger S. Development of physical dependence on morphine in respect to time and dosage and quantification of the precipitated withdrawal syndrome in rats. Psychopharmacologia (Berl.) 1973; 33:19-38.
11. Neil BS, Sparber SB. Mianserin attenuates naloxone-precipitated withdrawal signs in rat acutely or chronically dependent upon morphine. PharmacolExpThera1986; 236(1):157-65.
12. Tuesta L,Fowler CD, Kenny PJ. Recent advances in understanding nicotinic receptor signaling mechanisms that regulate drug self-administration behaviour. BiochemPharmacol. 2011; 82(8): 984–95.
13. Janssen J, Lackman G. Nutmeg oil: identification and quantification of its most active constituents as inhibitors of platelet aggregation. J Ethnopharmacol 1990; 29:179-188.
14. El-Alfy A, Lisa Wilson, Mahmoud A. ElSohly,Ehab A Abourashed. Towards a Better Understanding of the Psychopharmacology of Nutmeg.Activities in the Mouse. Tetrad Assay J Ethnopharmacol 2009; 126(2): 280–286.
15. Demetriades AK, Wallman PD, McGuiness A, Gavalas MC. Low cost, high risk: accidental nutmeg intoxication. Emergency Medicinal Journal 2005; 22: 223–225.
16. Beyer J, Ehlers D, Maurer HH. 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. Therapeutic Drug Monitoring 2006; 28(4): 568–575.
17. Muchtaridi, AnasSubarnas, Anton Apriyantono, ResmiMustarichie. Identification of Compounds in the Essential Oil of Nutmeg Seeds (*MyristicafragransHoutt.)* That Inhibit Locomotor Activity in Mice. Int. J. Mol. Sci. 2010; 11: 4771-4781.