WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 1, 329-337. Research Article ISSN 2277– 7105

EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF VARIOUS SOLVENT EXTRACTS OF *MYRISTICA FRAGRANS* SEEDS

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Article Received on
16 Nov. 2018,

Revised on 06 Dec. 2018,
Accepted on 27 Dec. 2018

DOI: 10.20959/wjpr20191-13988

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ABSTRACT

Background. *Myristica fragrans* (Houtt.), belonging to the family Myristaceae, is an evergreen tree whose fruits yield two spices, namely nutmeg and mace. Mace, which is the aril of the fruit, is used in Indonesian traditional medicine as an analgesic. Methanol extract of mace has been shown to be anti-inflammatory. Ethanol extract of the seed (nutmeg) has also been shown to be anti-inflammatory. It was the objective of the present study to determine the analgesic and anti-inflammatory activity of various solvent extracts of whole fruits (nutmeg plus mace) in rodent models. **Methods:** Anti-inflammatory activity of the various extracts was determined by measuring inhibition of carrageenan-induced inflammation of rat's hind paw. Peripheral analgesic activity was determined through inhibition of intraperitoneally injected acetic acid-induced writhings in mice. **Results:** In anti-inflammatory tests, crude methanol extracts and subsequent petroleum ether, n-hexane and chloroform fractions at doses of 200 and 400 mg per kg body weight inhibited carrageenan-induced paw edema, respectively, by 88.9, 90.9, 82.7, 86.1, 76.9, 87.5, 79.8, and 88.4%. By comparison, a standard drug, diclofenac sodium, when administered at a dose of 100 mg per kg reduced paw edema by 92.3% compared to control rats. Thus all the solvent fractions of *Myristica fragrans* fruit demonstrated considerable anti-inflammatory activity. In analgesic activity tests, the above fractions at the same two doses inhibited the number of writhings by

52.6, 57.8, 58.5, 60.7, 40.7, 57.0, 46.7, and 53.3%, respectively. Diclofenac sodium, which is also a standard analgesic drug, reduced the number of writhings by 76.3%. Thus the various extracts possessed analgesic activities but were not effective as diclofenac sodium. **Conclusion:** The various solvent extracts can be used as anti-inflammatory and analgesic agents and merit potential for further research.

KEYWORDS: Anti-inflammatory, analgesic, *Myristica fragrans*, pain.

BACKGROUND

Inflammation and pain can be acute or chronic, lasting for a few days or years, and resulting from simple causes as a fall or sprain, or associated with complicated disorders like rheumatoid arthritis or cancer. Pain is a common phenomenon, being experienced by possibly millions of people throughout the world if not billions at least once or twice a year. Available drugs other than opioid drugs (which are addictive) mainly are aspirin and acetaminophen. Prolonged use or over-dosage of the former can lead to gastric ulceration[1]; aspirin is also a blood thinning agent, and while it is prescribed after stroke, the drug can prolong bleeding time after cuts and wounds.[2] Acetaminophen, on the other hand, can cause hepatotoxicity.[3] Thus, new drugs to treat pain and inflammation are necessary and preferably the new drugs should not have any adverse side-effects.

Plants have always formed a source of new drugs.[4] Ongoing research activity throughout the world is identifying potential plants and phytochemicals, which can be used as source for lead compounds and new analgesic and anti-inflammatory drugs.[5-7] We had been screening for analgesic plants for a number of years.[8-19] It was the objective of the present study to evaluate the anti-inflammatory efficacy of various solvent extracts of *Myristica fragrans* seeds in carrageenan-induced paw edema in rats, and analgesic efficacy in intraperitoneally injected acetic acid-induced abdominal writhings (constrictions) in mice.

METHODS

Plant material collection

Myristica fragrans seeds were collected from an herbal shop at Dhaka. The seeds were washed properly and then air dried for several days. The seeds were then grounded into a coarse powder using high capacity grinding machine.

Preparation of methanolic extract of grounded seeds

For preparation of methanol extract, 800g of the powder was extracted with 2.5 liters of methanol over 15 days with occasional stirring and shaking. The mixture was then filtered and methanol in the filtrate evaporated using a Rota evaporator. The final weight of the methanolic extract was 160g.[20]

Solvent-solvent partitioning

10g of crude methanolic extract was dissolved in 10% aqueous methanol and sequentially extracted with petroleum ether, n-hexane and finally with chloroform.[21]

Chemicals and Drugs

All chemicals and reagents were of analytical grade and obtained from local importers of Sigma Chemical Company, USA. Diclofenac sodium was obtained from Square Pharmaceuticals, Bangladesh.

Animals

Long-Evans rats of either sex were used for carrageenan-induced rat paw edema experiments. Rats weighed between approximately 80 to 100g. Swiss albino mice of both sexes, which weighed between 30 to 35g were used in the present study for acetic acid-induced writhing experiments. The animals were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The animals were housed in the Animal House of Nutrition and Food Department of the University of Dhaka and acclimatized for three days prior to actual experiments. During this time, the animals were fed with rat or mice chow (supplied by ICDDR,B) and water *ad libitum*. The study was conducted following approval by the Institutional Animal Ethical Committees of the University of Development Alternative and the University of Dhaka, Dhaka, Bangladesh.

Carrageenan-induced rat paw edema test for anti-inflammatory activity

The animals were weighed and randomly divided into 10 groups of 5 rats in each group. Each group received a particular treatment as shown in Table 1. Diclofenac sodium at 100 mg per kg body weight was used as the standard anti-inflammatory agent. After 30 mins of oral administration of extract or diclofenac, 0.1 ml of 1% carrageenan solution (w/v) was injected into the sub-plantar surface of the right hind paw of each rat in each group. The paw volume was measured by plethysmometer (UgoBasile, Italy)[22] at 1, 2, 3 and 4 hours after 30 minutes of carrageenan injection. Mean increases in paw volume were noted for the respective time

intervals; thus edema volumes in control [(C_t – C_o) control] and in groups treated with test materials [(C_t – C_o) treated] were calculated. Percentage inhibition of paw edema was calculated by using the following formula, as described below.

% paw edema inhibition = [{(C_t – C_o) control – (C_t– C_o) treated}/(C_t – C_o) control] X 100, where C_o = paw volume at zero time (before carrageenan injection), C_t = paw volumes at t time, (C_t – C_o) = paw edema.

Acetic acid-induced abdominal writhing test for analgesic activity

Fifty experimental animals (Swiss albino mice) were randomly selected, weighed and divided into ten groups consisting of 5 mice in each group. Each group received a particular treatment as shown in Table 3. Briefly, Group 1 mice were given vehicle and served as control, Group 2 mice were administered diclofenac sodium, while rest of the Groups received various solvent extracts. After forty minutes following administration of extract, vehicle or diclofenac sodium, glacial acetic acid (0.7% in water, v/v) was administered intraperitoneally to each animal of all the groups at a dose of 0.1 ml per 10g body weight, the method being a slight modification of a method as described earlier.[23]

Following administration of acetic acid, five minutes were given for the acid to induce full effect. The number of writhings was then counted for 10 minutes. Full writhing was occasionally not seen; writhing may start in an animal but not completed. These partial writhings were taken as half writhings and two half writhings were counted as a full writhing.

Statistical analysis

Experimental values are expressed as mean ± SEM. The results were analyzed statistically by one way analysis of variance (ANOVA) followed by Dunnett's test using SPSS ver. 17. A value of $P< 0.05$ was considered to be statistically significant.[20]

RESULTS

In anti-inflammatory tests, crude methanol extracts and subsequent petroleum ether, n-hexane and chloroform fractions at doses of 200 and 400 mg per kg body weight inhibited carrageenan-induced paw edema, respectively, by 88.9, 90.9, 82.7, 86.1, 76.9, 87.5, 79.8, and 88.4%. By comparison, a standard drug, diclofenac sodium, when administered at a dose of 100 mg per kg reduced paw edema by 92.3% compared to control rats. Thus all the solvent fractions of *Myristica fragrans* seeds demonstrated considerable anti-inflammatory activity.

The mean paw volume of rats receiving different extracts of *Myristica fragrans* seeds is shown in Table 1, while the percent inhibition of paw edema is shown in Table 2.

In analgesic activity tests, crude methanol extracts and subsequent petroleum ether, n-hexane and chloroform fractions at doses of 200 and 400 mg per kg body weight inhibited the number of writhings by 52.6, 57.8, 58.5, 60.7, 40.7, 57.0, 46.7, and 53.3%, respectively. Diclofenac sodium, which is also a standard analgesic drug, reduced the number of writhings by 76.3%. Thus the various extracts possessed analgesic activities but were not effective as diclofenac sodium. The results are shown in Table 3.

Table 1: Mean paw volume of rats receiving different extracts of *Myristica fragrans.*

Group	Dose	Mean paw volume (ml) ± SEM*			
		1st hour	2nd hour	3rd hour	4th hour
1) Control		0.410 ± 0.015	0.586 ± 0.014	0.656 ± 0.017	0.678 ± 0.015
2) Standard (Diclofenac Sodium)	100 mg/ Kg	0.364 ± 0.009*	0.398 ± 0.006*	0.376 ± 0.030*	0.298 ± 0.010*
3) Crude Methanolic Extract	200 mg/ Kg	0.350 ± 0.009*	0.394 ± 0.034*	0.364 ± 0.041*	0.304 ± 0.038*
4) Crude Methanolic Extract	400 mg/ Kg	0.352 ± 0.010*	0.392 ± 0.010*	0.326 ± 0.009*	0.300 ± 0.007*
5) Petroleum Ether fraction	200 mg/ Kg	0.386 ± 0.013*	0.432 ± 0.010*	0.426 ± 0.022*	0.362 ± 0.019*
6) Petroleum Ether fraction	400 mg/ Kg	0.342 ± 0.007*	0.344 ± 0.020*	0.352 ± 0.019*	0.306 ± 0.017*
7) n-Hexane fraction	200 mg/ Kg	0.358 ± 0.023*	0.390 ± 0.022*	0.398 ± 0.022*	0.358 ± 0.020*
8) n-Hexane fraction	400 mg/ Kg	0.368 ± 0.010*	0.386 ± 0.023*	0.382 ± 0.019*	0.322 ± 0.022*
9) Chloroform fraction	200 mg/ Kg	0.348 ± 0.009*	0.396 ± 0.020*	0.402 ± 0.020*	0.338 ± 0.011*
10) Chloroform fraction	400 mg/ Kg	0.390 ± 0.010*	0.444 ± 0.013*	0.396 ± 0.024*	0.346 ± 0.024*

All administrations were made orally. Values represented as mean ± SEM, (n=5); *$P < 0.05$ versus Control

Table 2: Paw edema and percent inhibition of paw edema at different time intervals with different solvent extracts.

Group	Dose	Paw edema (ml)				% Paw edema inhibition			
		1st hour	2nd hour	3rd hour	4th hour	1st hour	2nd hour	3rd hour	4th hour
1) Control		0.148	0.324	0.394	0.416				
2) Standard (Diclofenac Sodium)	100 mg/ Kg	0.098	0.132	0.110	0.032	33.784	59.259	72.081	92.308
3) Crude Methanolic Extract	200 mg/ Kg	0.092	0.136	0.106	0.046	37.838	58.025	73.096	88.942
4) Crude Methanolic Extract	400 mg/ Kg	0.090	0.130	0.064	0.038	39.189	59.877	83.756	90.865
5) Petroleum Ether fraction	200 mg/ Kg	0.096	0.142	0.136	0.072	35.135	56.173	65.482	82.692
6) Petroleum Ether fraction	400 mg/ Kg	0.094	0.096	0.104	0.058	36.486	70.370	73.604	86.058
7) n-Hexane fraction	200 mg/ Kg	0.096	0.128	0.136	0.096	35.135	60.494	65.482	76.923
8) n-Hexane fraction	400 mg/ Kg	0.098	0.116	0.112	0.052	33.784	64.198	71.574	87.500
9) Chloroform fraction	200 mg/ Kg	0.094	0.142	0.148	0.084	36.486	56.173	62.437	79.808
10) Chloroform fraction	400 mg/ Kg	0.092	0.146	0.098	0.048	37.838	54.938	75.127	88.462

Table 3: Analgesic activity of crude methanolic extract and its different fractions of *Myristica fragrans* seeds.

Group	Dose	Number of writhings Mean ± SEM	% inhibition of writhing
1) Control		100.0 ± 0.7	-
2) Standard (Diclofenac sodium)	100 mg/ Kg	23.7 ± 0.5	76.3*
3) Crude Methanolic Extract	200 mg/ Kg	47.4 ± 1.0	52.6*
4) Crude Methanolic Extract	400 mg/ Kg	42.2 ± 1.1	57.8*
5) Petroleum Ether fraction	200 mg/ Kg	41.5 ± 0.6	58.5*
6) Petroleum Ether fraction	400 mg/ Kg	39.3 ± 0.7	60.7*
7) n-Hexane fraction	200 mg/ Kg	59.3 ± 0.7	40.7*
8) n-Hexane fraction	400 mg/ Kg	43.0 ± 1.1	57.0*
9) Chloroform fraction	200 mg/ Kg	53.3 ± 0.9	46.7*
10) Chloroform fraction	400 mg/ Kg	46.7 ± 1.1	53.3*

All administrations were made orally. Values represented as mean ± SEM, (n=5); *$P < 0.05$; significant compared to control animals.

DISCUSSION

Preliminary analysis of seed extracts has confirmed the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, phenols, anthraquinones, cardiac glycosides, coumarins, anthocyanin, chalcones, emodins, and triterpenoids [24]. Emodin from *Ventilago leiocarpa* has been reported to have anti-inflammatory effect [25]. Alkaloids, flavonoids or triterpenoids may also be responsible for the observed analgesic and anti-inflammatory effects. Such effects of this group of compounds have been described earlier [26-28]. However, the exact identification of component(s) in *Myristica fragrans* seeds responsible for the analgesic and anti-inflammatory effects remain to be elucidated.

CONCLUSION

The results suggest that various extracts of seeds of *Myristica fragrans* can be used as anti-inflammatory and analgesic agents.

Conflicts of interest

The author(s) declare that they have no competing interests.

REFERENCES

1. Cryer B, Mahaffey KW. Gastrointestinal ulcers, role of aspirin, and clinical outcomes: pathobiology, diagnosis, and treatment. J Multidiscip Healthc, 2014; 7: 137-146.

2. Stroke Association. Blood-thinning medication after stroke. Stroke Association Factsheet 11, version, 2015; 1.1: 8.
3. Yoon E, Babar A, Choudhary M, Kutner M, Pyrsopoulos N. Acetaminophen-induced hepatotoxicity: a comprehensive update. J ClinTransl Hepatol, 2016; 4: 131-142.
4. Shakya AK. Medicinal plants: Future source of new drugs. Int J Herbal Med, 2016; 4: 59-64.
5. Eliaser EM, Ho JH, Hashim NM, Rukayadi Y, Ee GCL, Razis AFA. Phytochemical Constituents and Biological Activities of *Melicope lunu-ankenda*. Molecules, 2018; pii: E2708.
6. De Souza GR, De-Oliveira ACAX, Soares V, Chagas LF, Barbi NS, Paumgartten FJR,da Silva AJR. Chemical profile, liver protective effects and analgesic propertiesof a *Solanum paniculatum* leaf extract. Biomed Pharmacother, 2018; 110:129-138.
7. Ajaib M, Ishtiaq S, Siddiqui MF. Comparative analgesic evaluation of *Himalrandia tetrasperma* and *Wendlandia exserta* of family Rubiaceae after induction of pain in mice. Pak J Pharm Sci, 2018; 31: 2509-2514.
8. Haque ME, Rahman S, Rahmatullah M, Jahan R. Evaluation of antihyperglycemic and antinociceptive activity of *Xanthium indicum* stem extract in Swiss albino mice. BMC Complement Alternat Med., 2013; 13: 296-299.
9. Haque AKMM, Kabir MZ, Rahman S, Rahman MM, Jahan R, Hossan MS, Rahmatullah M. Preliminary phytochemical screening, oral glucose tolerance, analgesic and acute toxicity studies with *Dendrocalamus giganteus* aerial parts. J Chem Pharm Res., 2014; 6: 397-402.
10. Rahmatullah M, Hosain M, Rahman S, Rahman S, Akter M, Rahman F, Rehana F, Munmun M, Kalpana MA. Antihyperglycaemic and antinociceptive activity evaluation of methanolic extract of whole plant of *Amaranthus tricolour* L. (Amaranthaceae).Afr J Trad Complement Altern Med, 2013a; 10: 408-411.
11. Rahmatullah M, Hossain M, Mahmud A, Sultana N, Rahman SM, Islam MR, Khatoon MS, Jahan S, Islam F. Antihyperglycemic and antinociceptive activity evaluation of 'khoyer' prepared from boiling the wood of *Acacia catechu* in water. Afr J Trad Complement Altern Med, 2013b; 10: 1-5.
12. Akter M, Mitu IZ, Proma JJ, Rahman SM, Islam MR, Rahman S, Rahmatullah M. Antihyperglycemic and antinociceptive activity evaluation of methanolic extract of *Trichosanthes anguina* fruits in Swiss albino mice. Adv Nat ApplSci, 2014; 8: 70-74.

13. Hossain AI, Faisal M, Rahman S, Jahan R, Rahmatullah M. A preliminary evaluation of antihyperglycemic and analgesic activity of *Alternanthera sessilis* aerial parts. BMC Complement Alternat Med, 2014; 14:169-173.
14. Jahan S, Rahmatullah M. Methanolic extract of aerial parts of *Raphanus sativus* var. *hortensis* shows antihyperglycemic and antinociceptive potential. World J Pharm PharmSci, 2014; 3:193-202.
15. Irin S, Bappy MS, Neesa L, Rahmatullah M. Preliminary phytochemical analysis and antinociceptive activity studies with methanol extract of whole plants of *Senna obtusifolia*. World J Pharm Res, 2017; 6: 124-130.
16. Rahman MM, Khanom SI, Sarkar SC, Rahmatullah M. Antinociceptive activity evaluation of *Momordica charantia* L. fruit methanolic extract. World J Pharm Pharm Sci, 2017; 6: 173-178.
17. Nipu AH, Akter S, Rahmatullah M. Preliminary phytochemical screening and evaluation of antihyperglycemic and antinociceptive effects of a combination of *Leucas aspera* aerial parts and *Zingiber officinale* rhizomes. World J Pharm Pharm Sci, 2017; 6: 135-145.
18. Akter F, Rahmatullah M.Antinociceptive activity evaluation of leaves of *Malva verticillata* L. J Med Plants Stud, 2018; 6: 152-154.
19. Mostafa MN, Islam MH, Rahmatullah M. Antinociceptive activity evaluation of corms of *Colocasia esculenta* var *esculenta*. World J Pharm Res., 2018; 7: 165-171.
20. Bhuiyan MSA, Islam MS, Ganguly A, Al-Mahmud MZ, Rashid MM, Ayubee MS, Akhter F, Rahmatullah M. Evaluation of oral glucose tolerance efficacy of various solvent extracts of *Myristica fragrans* seeds. World J Pharm Res, 2018; 7: 167-173.
21. Beckett M, Stenlake OI. *In*: Chromatography. Practical Pharmaceutical Chemistry. 3rd Edition, 1986; 2: 75-76.
22. Hafeez A, Jain U, Sajwan P, Srivastava S, Thakur A. Evaluation of carrageenan induced anti-inflammatory activity of ethanolic extract of bark of *Ficus virens* Linn. in Swiss albino mice. J Phytopharmacol, 2013; 2: 39-43.
23. Mishra D, Ghosh G, Kumar PS, Panda PK. An experimental study of analgesic activity of selective COX-2 inhibitor with conventional NSAIDs. Asian J Pharm Clin Res., 2011; 4: 78-81.
24. Thomas RA, Krishnakumari S. Phytochemical profiling of *Myristica fragrans* seed extract with different organic solvents. Asian J Pharm Clin Res., 2015; 8: 303-307.

25. Chang CH, Lin CC, Yang JJ, Namba T, Hattori M. Anti-inflammatory effects of emodin from *Ventilago leiocarpa*. Am J Chin Med., 1996; 24: 139-142.
26. Muhammad N, Shrestha RL, Adhikari A, Wadood A, Khan H, Khan AZ, Maione F, Mascolo N, De Feo V. First evidence of the analgesic activity of govaniadine, an alkaloid isolatedfrom *Corydalis govaniana* Wall. Nat Prod Res., 2015; 29: 430-437.
27. Shah M, Parveen Z, Khan MR. Evaluation of antioxidant, anti-inflammatory, analgesic and antipyretic activities of the stem bark of *Sapindus mukorossi*. BMC Complement Altern Med., 2017; 17: 526.
28. Aati HY, El-Gamal AA, Kayser O, Ahmed AF. The Phytochemical and Biological Investigation of *Jatropha pelargoniifolia* Root Native to the Kingdom of Saudi Arabia. Molecules, 2018; 23: pii, E1892.