Phytochemical and Pharmacological Evaluation of Myristica fragrans Houtt (Myristicaceae): A Comprehensive Review

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ABSTRACT

Objective: *Myristica fragrans* Houtt commonly used as traditional medicine for alleviating of various disorders. The purpose of our study was to map out the *in vitro* antioxidant property and *in vivo* anti-hyperglycemic and analgesic effect of the methanolic extract of *Myristica fragrans* Houtt. (Seed and mace) (Myristicaceae) on Swiss albino mice. Methods: The processed powder of *Myristica fragrans* Houtt (seed and mace) were subjected to methanolic extraction by soxhlet filtration methods, and the desiccated extract was used for screening of antioxidant by DPPH free radical scavenging assessment as well as total phenolic content by using folin-ciocalteu reagent.Anti-hyperglycemic effect and analgesic action tested through alloxan induced antidiabetics test and acetic acid-tempted writhing test on mice. Results: In DPPH free radical scavenging assessment, free radicals neutralization expressed as % of inhibition 49.69±0.06% also by IC_{50} values as 68.43 µg/ml surmise middle level of antioxidant property. The total phenolic content expressed as 186.25 mg/g equivalent of gallic acid indicates, active phenolic content. Oral administration of 200 and 400 mg/kg of extract dose and reference drug vildagliptin (50 mg/kg) for the duration of the 4-day study period, and initiated % of inhibition the blood glucose level measured as 22.48%, 44.78% and 62.02% regard as the significant anti-hyperglycemic properties. The analgesic activity was investigated by using the acetic acid-induced writhing test in mice, at the dose of 200 mg/kg body and 400mg/kg weight, and resulting 50.4% and 68.10% correspondingly, which was considerably significant with a standard drug. Conclusion: The present study suggests that methanolic extract of seed and mace of Myristica fragrans Houtt can manage moderate oxidative stress as well as perform the painkilling action. Besides, prolong medication may enhance the new dimension of anti-hyperglycemic activity.

Keywords: Antioxidant, Total Phenolic content, *Myristica fragrans* Houtt as (Mf_{sm}), Analgesic activity, Writhing test, Anti-hyperglycemic test.

INTRODUCTION

Which components can promote health and alleviate illness manifested as “Medicine” and medicine belongs to chemical compounds. The large diversified chemical compounds like phytochemicals (alkaloids, flavonoids, tannins, and phenolic) constituents are present in plants, which are biologically active to show pharmacological effects. Such chemical substances can act as an originator for synthesis drugs.For reducing demoralizing illness with significant morbidity and mortality, the medicinal plant considered as a safe remedy (cardiovascular problems, cancer, liver disorders, central nervous system, digestive and metabolic disorders) and chemopreventive actions and costs effectiveness, less toxic[1,2,3-8,9.]

In this phytochemical and pharmacological assay, *Myristica fragrans* Houtt *(Mf_{sm})* (seed and mace) tested for their oxidative stress inhibitory effect, analgesic, and antihyperglycemic effect. *Myristica fragrans* Houtt.(Nutmeg, mace –English; Jai phal –India; Seed and mace- Jai phalJaiyatri Bangladesh; Pala -Indonesian) Belonging to the family of Myristicaceae is a scented evergreen tree that grows 30–39 ft (1 ft=30.48 cm) high with spreading branches and yellow fleshy fruits, having an appearance like apricot or peach. Both the seed (Nutmeg) and its fleshy aril (mace) are known as spices[10]. From the ancient time, it used as a traditional medicine for the remedial purpose of a broad range of disease. Nutmeg useful for curing Diarrhea, mouth sore and insomnia and inhibitory activity against several kinds of anaerobic and aerobic microorganisms[11]. Besides,*Myristica fragrans* still used as, antioxidant, analgesics, stomachic’s, aphrodisiacs, digestives, hypnotics; amenorrheal agents[12,13]. The significant reducing capability of bacterial infection[14] and as an aphrodisiac, anti-rheumatoid, anti-malarial, stimulant, and post childbirth[15] also reported.From the experimental evidence, *Myristica fragrans* Houtt exhibit potent antifungal and antibacterial (the majority of gram positive and gram negative) activities[16,17-19]. At the same time, *Myristica fragrans* Houtt used as food preservatives[20], antiseptic and disinfectant[21]. In another study, confirmed that nutmeg extract had significant prevention capability of hypercholesterolemia and atherosclerosis[22] and hepatoprotective activity[23]. Even it has proven high anti-inflammatory action[24,25-27] and antidepressant effect[28].

Thus, we studied the methanolic extract of *Myristica fragrans* Houtt*(Mf_{sm})* seed and mace for it antioxidant property, along with analgesic and antidiabetic effects.

MATERIALS AND METHODS

Chemicals and solvents

DPPH (2, 2-Diphenyl-1-picrylhydrazyl) was obtained from Sigma Chemical Co., USA. Methanol, ascorbic acid,folin-acid, folin-ciocalteu/folins phenol reagent, sodium carbonate (Na2CO3), gallic acid, alloxan from (Fluka, Germany), vildagliptin-50mg/tablet collected from drug manufacturer - Novartis, Bangladesh; and glucose

estimation kit (Human, Germany). All the chemicals used, including the solvents, were of analytical grades.

Instruments

HACH DR 4000U UV-visible spectrophotometer equipped with quartz cells of the 1-cm light path used to determine the molecular absorption spectra and absorbance at specific wavelengths.

Collection of the plant parts

The fresh, dried seed and mace of *Myristica fragrans* Houtt *(Mf_{sm})* were collected from a local savory spice shop, Mirpur, Dhaka, as well as from Sylhet district, Bangladesh. Shapna Sultana, Lecturer, Department of Pharmacy, Southeast University, Banani, primarily identified the collected, dried seed and mace. As a final point of attribution by a taxonomist at "Bangladesh National Herbarium" Mirpur, Dhaka, Bangladesh a receipt specimen was deposited in the herbarium division numbered as DACB (Accession Number 43646).

Drying, pulverization, preservation of plant parts The collected seed and mace were washed with fresh water and dried for one week. The seed and mace collectively grounded into a coarse powder with the help of a suitable grinder machine and passing through a sieve, in pharmaceutical technology lab, Southeast University, Dhaka. As a final point, crushed powder stored in a cool, dark, and dry place in an airtight container, and kept until analysis commenced.

Extraction of the plant material

By using soxhlet apparatus dried plant material were refluxed with methanol. The vapor flows through a coil where they condense back to a liquid which is then collected in the receiving vessel. The whole mixture was successively filtered through a piece of clean, white cotton material and Whatman filter paper (Bibby RE200, Sterilin Ltd., UK).

Experimental animals

The Swiss albino mice of both sexes weighing (29.3±3.9) were used to conduct the research and procured from the animal research branch of the (icddr,b-International Centre for Diarrhoeal Disease and Research) Bangladesh. They kept under standard husbandry conditions (temperature 23±2 C relative humidity 55± 10 and 12 light and 12-hour dark cycle).The animals fed with commercial diet pellets and water ad libitum. Before experimentation session, the animals were allowed to acclimatize to the atmosphere for seven days. Animals were kept fasting overnight but allowed free access to water.

Ethical approval

As per University requisition, icddr,b; provided the animals for experimenting purpose. All the testing on animals were performed by guidelines of the institutional animal ethics committee[29] Southeast University, Banani Dhaka, Bangladesh.

In vivo test

Antioxidant activity measured by DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay In a chemical reaction with dpph, antioxidants stabilized the free radical and reduced to dpph-H. As a consequence; the absorbance has decreased from the DPPH radical to the DPPH-H form. The scale of yellowing indicates the scavenging potential of the antioxidant (Hydrogen donating capacity) compounds[30]. The scavenging reaction between (DPPH) and plant extract [an antioxidant (H-A)] can be expressed as:

(DPPH) + (H-A) ----------DPPH-H + (A)
(Purple) (Extract) (Yellow)

The free-radical scavenging activity of *Myristica fragrans* Houtt *(Mf_{sm})* extracts was measured by a decrease in the absorbance of a methanol solution of DPPH[31]. A stock solution of DPPH (400 µg/ml) was prepared in methanol, and 100 µl of this stock solution was added to 5 ml of solutions of *Myristica fragrans* Houtt (Mf_{sm}) extracts of different concentrations (5, 10, 25 and 50 µg/ml)[32]. The solutions were then mixed properly and kept in the dark for 20 min, and the absorbances were measured at 517 nm by using a HACH DR 4000U UV-visible spectrophotometer. Scavenging activity was expressed as the percentage inhibition calculated using the following formula as, the percentage of inhibition = $[(A_0 – A_1) / A_0] \times 100$; Where, A_0 = Absorbance of the control; A1 = Absorbance of the plant extract/ standard. And the IC_{50} value was calculated from the equation of line obtained by plotting a graph of concentration (µg/ml) versus % inhibition[33]. Ascorbic acid used as a reference standard. The radical scavenging activity (RSA) or percentage inhibition evaluated by comparing the values of absorbance for the investigational samples and control following the equation as indicated[34].

Total phenolic content test

By using folin-ciocalteu reagent (FCR - a hetero-poly phosphotungstates-molybdates utilized for the colorimetric assay of phenolic and polyphenolic antioxidants), the total phenolic content of certain plant materials can be measured, which help to measure reducing capacity. Sequences of reversible one or two electron reduction reactions lead to blue species possibly $(PMoW_{11}O_{40})^4$. In essences, it is assumed that the molybdenum is easier to be reduced in the complex and electron transfer reaction occurs between reluctant and Mo (VI). The overall procedure performs by using folinciocalteu reagent[35] and aluminum chloride method[36,37] correspondingly. The content of phenolic in the extract of *Myristica fragrans* Houtt *(Mf_{sm})* was calculated from regression equation of the calibration curve and expressed as gallic acid standard /equivalent (GAE)[38]. *In vivo test*

Analgesic activity (Acetic acid-tempted writhing in mice): The analgesic activity of *Myristica fragrans* Houtt *(Mf_{sm})* assayed by the acetic acid induced writhing test[39]. One hour before peritoneal insertion of acetic acid 300 mg/kg (3% solution in sterile distilled water) i.p, diclofenac-Na used as reference standard drug and *Myristica fragrans* Houtt *(Mf_{sm})* extract at different concentration administrated orally. After 20 minutes of addition of acetic acid, the writhing of each counted and the percent of inhibition was calculated by comparing with control group. If the sample possesses analgesic activity, it will subordinate the number of writhing than the standard and revealed the analgesic activity as % inhibit of writhing. *Anti-hyperglycemic activity on alloxan induced diabetic mice*

To complete the antidiabetic activity, indiscriminately separated group of mice was injected intraperitoneally (I.P.) at a dose of 120 mg/kg b.w. Freshly prepared alloxan monohydrate (fluka, Germany). After an hour of alloxan administration, animals were fed ad libitum and 1ml of (100 mg/ml) glucose i.p. to combat ensuring severe hypoglycemia after 72 hr of alloxan injection; the animals were tested for evidence of diabetes by estimating their blood glucose level using a glucometer. Mice showed FBG > 150 mg/dl considered diabetic and selected for studies.

Animal grouping and experimental design Animals selected fasted overnight and then divided into four groups (n=4) as follows

Group-I**:** Normal control mice (non-alloxanized) that administered distilled water only; Group-II:diabetic control mice (untreated, alloxanized); Group-II: Diabetic mice administered with *Myristica fragrans* Houtt **(*Mf*$_{sm}$)** (200 mg/kg/day) respectively; Group-IV: Diabetic mice administered with *Myristica fragrans* Houtt **(*Mf*$_{sm}$)** (400 mg/kg/day) respectively; Group-V: mice administered once with vildagliptin (50 mg /kg) as reference standard drug.

Statistical Analysis

All the tests were performed triplicates and values are expressed as Mean ± Standard Deviation /SEM by using Microsoft Excel, 2007.

RESULTS

DPPH free radical scavenging activity

Methanolic extract of *Myristica fragrans* Houtt **(*Mf*$_{sm}$)** demonstrated proton /H-donor activity and showed potent scavenging rate of dpph. The percentage (%) of scavenging was found to be concentration dependent, i.e., scavenging capacity increases with the increase of the concentration of both the extracts. By contrasting % of inhibition for **(*Mf*$_{sm}$** and AA for the concentration of 80 µg/ml, found 49.69±0.06% and 88.22 ±0.10% (Table: 1 and Table: 2) respectively, which indicate the median antioxidant capacity of sample *Myristica fragrans* Houtt **(*Mf*$_{sm}$)**. Additionally, the IC_{50} values of **(*Mf*$_{sm}$)** and AA is 68.43 µg/ml. and 12.82 µg/ml calculated by using regression equation Y_{Mfsm} = (0.383x +23.79; R^2=0.575) and (Y_{AA} =0.659x+41.55; R^2=0.777) respectively. Furthermore, the % of inhibition depicted in Fig:1 reveals the insignificant difference of ***Mf***$_{sm}$ and AA.

Determination of total phenolic content

The total phenolic content in the extracts of *Myristica fragrans* Houtt **(*Mf*$_{sm}$)** determined according to the colorimetric folin-ciocalteu assay with gallic acid as a standard compound. The total phenolic content was calculated from regression equation curve (y=0.012x+0.342, R^2=0.929) and expressed as gallic acid equivalent (GAE) (Table:3 and Table:4).A potent range of total phenolic content was found in the plant materials to be186.25 mg/g plant extract (in GAE-Table 3) for *Myristica fragrans* Houtt **(*Mf*$_{sm}$)** (Table 4).

Calculation

Regression equation y=0.012x+0.342, R^2=0.929; y=0.789; m=0.012; c=0.342

x= 37.25 micro gm/ml; C= 0.0373 mg/ml;

V=1 ml; m=.0002

$$A = (C \times V)/m$$
$$= 186.25 \text{ mg/g Gallic Acid}$$

Where, A = Total phenol content, mg/g plant extract, in Gallic acid

C= the concentration of Gallic acid established from the calibration curve, mg/ml

V = the volume of extract, ml; m = the weight of pure plant methanolic extract, g

The amount of total phenol content of *Myristica fragrans* Houtt **(*Mf*$_{sm}$)** is 186.25 mg/g gallic acid (GA) Acid

Analgesic activity

Animal were divided into four groups (4 animals in each group), as group 1: Control (Saline water treated); group 2: Positive control (Diclofenac treated);group 3: *Myristica fragrans* Houtt (*Mf*$_{sm}$) 200mg/kg; group 4**:** *Myristica fragrans* Houtt (*Mf*$_{sm}$) 400mg/kg.After administration of the different dose of methanolic extract of *Myristica fragrans* Houtt (*Mf*$_{sm}$) and standard drug diclofenac as the positive control, the numbers of writhing reduced and ensured analgesic effects. By comparing the number of writhing with the untreated control group *Myristica fragrans* Houtt (*Mf*$_{sm}$) showed almost similar % of inhibition as 68.10% at the dose of 400mg/kg. (Table 5)

Antidiabetic activity on alloxan-induced diabetic mice

The consequences after chronic administration of *Myristica fragrans* Houtt **(*Mf*$_{sm}$)** showed meaningful antihyperglycemic action between investigational and diabetic control mice. At a dose of 200 mg/kg body weight, *Myristica fragrans* Houtt **(*Mf*$_{sm}$)** significantly lowered blood glucose level and showed reduction of 22.48 % while at 400 mg/kg *Myristica fragrans* Houtt **(*Mf*$_{sm}$)** body weight dose, produced maximum reduction of 44.78% of blood glucose level, respectively, inhibition of blood glucose level 62.01 % was found for vildagliptin (50 mg /kg) on day 4 as a peak. (Table 6).

DISCUSSION

In vitro antioxidant activity

In vitro findings indicated that *Myristica fragrans* Houtt **(*Mf*$_{sm}$)** recognized their anti-oxidative abilities regarding

Table 1: The percentage of inhibition of methanolic extracts of *Myristica fragrans* Houtt (*Mf*$_{sm}$).

ition		Avg %	SEM	IC_{50}
0.000	0.000	0.00	0.00	
10.636	10.680	10.56	0.10	
31.964	32.378	32.11	0.13	68.43
42.037	39.854	41.66	0.96	
44.232	44.744	44.42	0.16	
49.690	49.578	49.69	0.06	

Conc.	Abs 1	Abs 2	Abs 3	% of inhib
0	1.775	1.777	1.779	0.000
5	1.591	1.588	1.589	10.366
10	1.207	1.209	1.203	32.000
20	1.010	1.030	1.070	43.099
40	0.989	0.991	0.983	44.282
80	0.891	0.894	0.897	49.803

Note: All values of absorbance are mean ± SEM of triplicates.IC_{50} (regression equation calculated the inhibitory concentration by half or 50%).

Table 2: The percentage of inhibition of ascorbic acid (AA).

Conc.	Abs 1	Abs 2	Abs 3	% of inhibition			AVG%	SEM	IC_{50}
0	1.785	1.810	1.779	0.000	0.000	0.000	0.00	0.00	
5	1.281	1.279	1.265	28.235	29.337	28.893	28.82	0.32	
10	0.839	0.832	0.827	52.997	54.033	53.513	53.51	0.30	12.82
20	0.669	0.671	0.674	62.521	62.928	62.114	62.52	0.24	
40	0.419	0.401	0.418	76.527	77.845	76.504	76.96	0.44	
80	0.209	0.211	0.213	88.291	88.343	88.027	88.22	0.10	

Note: All values of absorbance are mean±SEM of triplicates.IC_{50} (regression equation calculated the inhibitory concentration by half or 50%).

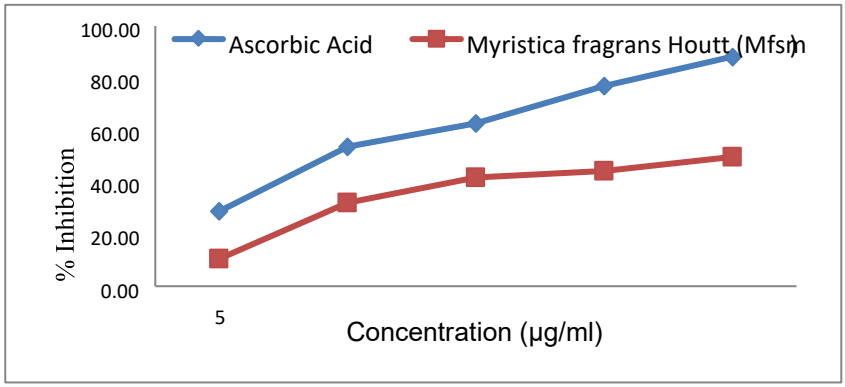

Figure 1: The antioxidant activity of methanolic extracts of *Myristica fragrans* Houtt (*Mf*$_{sm}$) and standard ascorbic acid (AA) determined by using DPPH method.

Table 3: Absorbance of gallic acid at different concentration.

Conc.	Abs	Abs	Abs	Avg
200	2.890	2.870	2.930	2.897
150	2.110	2.190	2.170	2.157
100	1.390	1.420	1.450	1.420
50	1.021	1.040	0.990	1.017

Note: All absorbance presented as Abs and concentration as Conc.

Table 4: Absorbance of methanolic extract of *Myristica fragrans* Houtt (*Mf*$_{sm}$).

Total phenolic content

Myristica fragrans Houtt (*Mf*$_{sm}$)

Conc.	Abs	Abs	Abs	AVG	ST. DE
0.0002	0.781	0.797	0.789	0.789	0.008

Note: All absorbance presented as Abs and concentration as Conc.

DPPH radical scavenging activity and total phenolic content.

DPPH radical scavenging activity

Myristica fragrans Houtt traditionally used as medicinal plant over the decades. Many experimental studies have reported that the methanolic/acetone extract of nutmeg seed showed good antioxidant activity by methods of 1,1diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) due to high content of tannin,flavonoids and terpenoids[40,41-44]. The first objective of this work was to evaluate the phytochemicals composition on the ***Mf$_{sm}$*** .In the present study, the ***Mf$_{sm}$*** confirmed good antioxidant properties as IC_{50} values, contrast with AA is 12.82 µg/ml and 68.43 µg/ml correspondingly. The obtained result demonstrated that the *Mf$_{sm}$* showed an important ability to donate a proton or neutralizing capacity of free radicles such as DPPH radicles with % of inhibition 49.69±0.06% compared with AA (Ascorbic acid) 88.22 ± 0.10% at a concentration of 80 µg/ml.

According to the experimental assay of Srinivasan*et al.*, 2005& Gupta *et al*. 2013 [45-46] spices possession as medicinal properties and our finding may anticipate the ability to care for the consumer's health from various free radical-related diseases.

Total phenol content

The second purpose of this study was to evaluate the phenolic compound by considering Plants are the primary source of the phenolic compound[47]. Many studies focused on the correlation of antioxidant activity to phenolic compounds content. The results of Kumar *et al.*[48] Yao *et al.*[49] and Hinnenburg *et al.*[50] stated a strong correlation between phenol and antioxidant activity.

Proceeding randomized experiments show different types of phenolic compounds, which a hydroxyl group attached to the benzene ring and reveal as an active antioxidant[51,52]. Through our evaluation, we found the amount of total phenol content of *Myristica fragrans* Houtt **(*Mf$_{sm}$*)** is 186.25 /g Gallic Acid, which indicates potent phenolic content and helps to initiate the anti-hyperglycemic test and analgesic test. *In vivo anti-diabetic effect*

The previous randomized clinical trial demonstrated that *Myristica fragrans* Houtt can reduce the blood sugar level reduced significantly in alloxan induced diabetic rats as well as increase the insulin secretion[53]. Also, *Myristica fragrans* Houtt (seed) possess a significant dual agonist PPAR α/γ that ensure the potential anti-diabetic agent for the treatment of type 2 diabetes[54].

Table 5: Analgesic activity of methanolic extract of *Myristica fragrans* Houtt (*Mf$_{sm}$*) on mice presented.

Group Number of writhing	Mean	SD	SEM	Mean ±SEM	% of Inhibition	m1	m2	m3	m4
Control	32	31	29	33	31.25	1.71	0.85	31.25±0.85	00.00
Positive control	8	7	9	7	7.75	0.96	0.48	7.75±0.48	73.28
G -1(*Mf$_{sm}$*)200mg/kg	16	14	17	15	15.5	1.29	0.65	15.5±0.65	50.4
G-2 (*Mf$_{sm}$*) 400mg/kg	9	10	7	11	9.25	1.71	0.85	9.25±0.85	68.10

Note: All values of writhing are mean±SEM (n=4) .The percentage of inhibition of writhing in acetic acid induced mice.

Table 6: Blood glucose level of alloxan-induced diabetic mice after treatment with the methanolic extracts of *Myristica fragrans* Houtt (***Mf$_{sm}$***) 200mg/kg and 400mg/kg -body weight for consecutive four (04) days.

	M1	M2	M3	M4	Mean	SD	SEM	% of Inhibition
	5.7	5.4	6	5.5	5.65	0.26	0.13	
	5.4	6.1	4.9	5.7	5.525	0.51	0.25	
	5.2	5.7	5.3	5.5	5.425	0.22	0.11	
Normal control	6.3	5.1	5.8	5.3	5.625	0.54	0.27	------
	13.4	13.9	15.2	14.9	14.35	0.84	0.42	
	12.6	13.3	13.5	14.7	13.52	0.87	0.44	
Alloxanized control	16.4	17.2	16.7	16.9	16.8	0.34	0.17	
mice-untreated	15.3	15.7	16.2	16.4	15.9	0.50	0.25	-----
	12.4	10.9	10.4	9.9	10.9	1.08	0.54	20.16
	12.9	11.1	10.8	10	11.2	1.22	0.61	22.48
Myristica fragrans	13.7	13.1	12.3	11.2	12.57	1.08	0.54	18.25
(*Mf$_{sm}$*) 200mg/kg Houtt	13.1	11.8	11.9	10.3	11.77	1.15	0.57	21.37
	12.6	10.6	9.1	7.4	9.93	2.21	1.11	41.27
	12.3	9.4	8.9	7.8	9.6	1.92	0.96	36.59
Myristica fragrans	13.4	10.3	9.2	7.4	10.08	2.52	1.26	44.78
(*Mf$_{sm}$*) 400mg/kg Houtt	12.7	9.8	8.5	7.6	9.65	2.22	1.11	40.16
	11.3	6.9	6.5	5.2	7.475	2.65	1.33	53.98
	12.5	7.2	5.9	5.1	7.675	3.33	1.67	59.2
Vildagliptin50mg/k	13.1	7.3	6.4	5.4	8.05	3.54	1.73	58.77
g	12.9	7.6	6.1	4.9	7.875	3.53	1.76	62.01

Note: The value of glucose level expressed as Mean ± SEM where N=4.Glucose level suppression expressed as a form of % of inhibition.

In our experiment type -1 (Insulin-dependent), diabetes study model has been developed to investigate the antihyperglycemic activity of *Myristica fragrans* Houtt **(*Mf$_{sm}$*)**. By using alloxan (a beta-cytotoxin)

insulinsecreting pancreatic β-cells destructed (a reactive oxygen species-dependent oxidative damage [55] and resulting in the diminished level of serum insulin[56]. In our study investigation vildagliptin, 50 mg/kg/day as standard drug and *Myristica fragrans* Houtt **(*Mf*$_{sm}$)** extract dose at 200 and 400mg/kg administrated and 62.01 %,22.48 % and 44.78%, inhibition the blood glucose level correspondingly on day four (04) as a peak. Chronic administration of ***Mf*$_{sm}$** in diabetic mice resulted in an effective lowering of blood glucose level suggesting that the extracts might possess insulin-like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis since alloxan treatment causes permanent destruction of β-cells.

Analgesic effect

The writhing test is a very active method for preliminary evaluation of anti-nociceptive activity and the % inhibition of writhing values obtained in animals using this test correlated with the analgesic doses in humans,[57] still it cannot indicate whether the effects result from central /or peripheral actions. The analgesic potential of the extract was shown by acetic acid test to be significant but was not accurate[58].

The acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids[59].

The acetic acid-induced writhing response is a painful procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells[60] acid-sensing ion channels[61] and the prostaglandin pathways[62].

The previous investigation showed that several nutmeg (*Myristica fragrans*) preparations are used to relief sprains, rheumatism and paralysis[63] with analgesics agents as similar to non-steroidal anti-inflammatory drugs[64]. In our study, the acetic acid induced writhing reflexes were used to explicate central and peripheral antinociceptive effects. The mean number of abdominal constriction after I.P. injection of acetic acid on mice compared after treatment with diclofenac sodium (50 mg/kg) and methanolic extract of *Myristica fragrans* Houtt (*Mf*$_{sm}$) at the dose of 200mg/kg and 400mg/kg. A dose-dependent reduction in the number of writhing was observed in different concentration and depicted as % inhibition of writhing response as 73.28%, 50.4% and 68.10% correspondingly.

CONCLUSION

The complete test indicates that *Myristica fragrans* Houtt (*Mf*$_{sm}$) have antioxidant potential as well as phenolic content property which help to manage the hyperglycemic condition by 40-43% and showed useful analgesic activity in acetic acid induced writhing method. Further studies are needed to spot out the bioactive structural association with the anti-hyperglycemic and analgesic activity.

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