EXPERIMENTAL INVESTIGATION OF ANTI-INFLAMMATORY AND ANTISPASMODIC PROPERTIES OF ETHANOLIC EXTRACT OF *MYRISTICA FRAGRANS HOUTT*. LEAVES

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***Abstract*:** The present study investigated the anti-inflammatory and antispasmodic potential of the ethanolic leaf extract of *Myristica fragrans Houtt*. Anti-inflammatory activity was evaluated using the protein denaturation assay, an established in vitro method for preliminary screening. The extract showed a concentration-dependent ability to inhibit protein denaturation, indicating effective anti-inflammatory activity. Antispasmodic effects were assessed through dose–response curve analysis by comparing the contractile responses induced by acetylcholine alone and in combination with atropine or the leaf extract. Acetylcholine produced pronounced smooth muscle contraction, whereas the presence of the extract significantly reduced the contractile response, demonstrating smooth muscle relaxation. The observed decrease in the dose–response curve suggests a spasmolytic effect, possibly mediated through interference with muscarinic receptor signalling or calcium-dependent contraction mechanisms. The combined findings indicate that *Myristica fragrans* leaves possess dual anti-inflammatory and antispasmodic activities. These pharmacological effects are likely associated with bioactive phytoconstituents such as flavonoids and phenylpropanoids, supporting the potential therapeutic value of the leaf extract in the management of inflammatory conditions and smooth muscle spasms.

***IndexTerms*-** *Myristica fragrans houtt.*, Ethanolic Leaf Extract, Anti-inflammatory Activity, Protien denaturation Assay, Antispasmodic Activity, Chick ileum.

I.INTRODUCTION

Inflammation is a complex biological reaction of vascular tissues to infections, injured cells, or irritants, Redness, edema, fever, pain, and loss of function are its hallmarks. General "flu-like" symptoms including fever, exhaustion, cold, appetite loss, and muscle rigidity can be linked to inflammation. It is well known that the inflammatory response is greatly influenced by the migration of leukocytes from the venous systems to the site of damage and the release of cytokines. The area becomes warmer and redder as a result of the increased blood flow caused by these substances. Certain drugs induce fluid to seep into the tissues, which leads to edema. As a result, inflammation can trigger nerves, resulting in pain, swelling, and redness. The complicated biological reaction to damaging stimuli, inflammation serves as the host's defence and is typically triggered in the majority of disease states, such as irritation, cell damage, and pathogen exposure.

Anti-inflammatory medications aim to reduce tissue damage and improve patient comfort by interfering with the pathophysiology of inflammation. Glucocorticoids and non-steroidal anti-inflammatory medicines (NSAIDs) are the two main families of anti-inflammatory agents. These are fundamentally different in how they work. To put it briefly, glucocorticoids work by blocking the production of prostaglandins and proteins that are involved in inflammatory processes. These proteins include corticosteroids, which are used to treat autoimmune inflammatory response and asthma, among other conditions. Contrarily, non-steroidal medications work by inhibiting the cyclooxygenase enzyme and are recommended for the treatment of moderate to mild pain as well as the regulation of body temperature. Acetylsalicylic acid is an example of a non-steroidal medication .

spasms are involuntary contractions that impede free and efficient voluntary muscle function. They are typically accompanied by pain. Spasm can result from a variety of illnesses and is frequently linked to stroke, multiple sclerosis, and spinal injuries. When spinal motor processes are not inhibited, spasticity and rigidity result. A muscle may cause a spasm in a number of situations: (i) motor axon depolarization that is unstable; (ii) muscle contractions that continue even when the muscle's innervation is normal and despite attempts at relaxation (myotonia); (iii) the muscle may decontract slowly following one or more contractions, as in hypothyroidism; and (iv) muscles lack the energy to relax .A spasm refers to a rapid, involuntary contraction occurring in a muscle, multiple muscles, or a hollow organ like the bladder.

Antispasmodic activity refers to a substance's capacity to relieve muscle spasms and stress in the smooth muscles of hollow organs and the muscular skeletal system. Nutmeg extract is used to alleviate the symptoms of cramping and discomfort caused by smooth muscles in the gastrointestinal and biliary tracts in a number of clinical situations. The aqueous extract of myristica scent relaxed guinea pig ileum and reduced the contraction rate generated by acetylcholine and histamine, indicating that the antispasmodic effect could be mediated by muscarnic and histamine receptors. The essential oil in nutmeg inhibits voltage-dependent calcium channels, modulates potassium channels, and regulates intracellular cAMP .

Myristica fragrans is a species belonging to the family Myristicaceae, a relatively small family of flowering plants that includes about 21 genera and approximately 500 species (Sauquet and Le Thomas). This family is composed primarily of shrubs and trees. Commonly known as nutmeg, *Myristica fragrans* is an aromatic evergreen tree indigenous to the eastern Moluccas Islands of Indonesia. Over time, its cultivation has spread to tropical regions such as Malaysia, Sumatra, Java, Sri Lanka, the West Indies, and

other similar climates (Purseglove et al., 1981). The plant is notable for producing two distinct spices: nutmeg, which is the seed kernel, and mace, the bright red aril or fleshy covering that surrounds the seed. Nutmeg trees are dioecious, meaning male and female flowers typically grow on separate trees, although occasionally both types may appear on the same plant (Everett, 1981). The flowers are small, pale yellow, and bell-shaped with three sepals. Also it reported to exhibit so many activity. Leafs contain many bioactive compounds including camphene, elemicin, eugenol, isoelemicin, isoeugenol, methoxyeugenol, pinene, sabinene, safrol, myristic acid, myristicin, and lignan were found in *M. Fragran* . Every part of the nutmeg plant, including leaves, stems, seeds, fruit flesh, and mace, contains various secondary metabolites, some of which have interesting bioactivities.

II.MATERIALS AND METHODS

2.1 Collection and authentification of plant material

Fresh *Myristica fragrans Houtt*. leaves were gathered from Ernakulam district, Kerala state and authentified from Department of Botany, Union Christian College, Aluva.

2.2 Processing of sample

In this procedure fresh leaves were collected, washed thoroughly with tap water followed by distilled water, and then shade-dried leaves for 20 days until constant weight was achieved. The dried materials were ground separately into coarse powder

2.3 Preparation of ethanolic extract .

Soxhlet extraction was carried out to obtain the ethanolic extract of *Myristica fragrans Houtt*. leaves. The dried leaf material was crushed into a coarse powder, and 30 g of the powder was introduced into the Soxhlet extractor. Extraction was performed using 300 mL of 70% ethanol at 60 °C for 16 hours. After the extraction period, the resulting extract was dried to yield the crude ethanolic leaf extract.

figure 1: soxhlation of *myristica fragrans houtt*

III. PHYTOCHEMICAL ANALYSIS .

Ethanolic extract of *Myristica fragrans Houtt. Leaves and pericarp* were subjected to phytochemical analysis

3.1 Test for cardiac glycoside

Keller Killani test

To the extract 5ml of water and 0.5 ml of strong lead acetate solution was added. Shake well and separate the filter. The filtrate was extracted with equal volume of chloroform. Then chloroform extract was evaporated to dryness and residue was dissolved in 3ml of glacial acetic acid followed by addition of few drops of FeCl3. The resulting solution was transferred to test tube containing 2ml of conc.sulphuric acid. A reddish-brown layer is formed which turns bluish green color after standing indicates the presence of cardiac glycoside.

Legal test

The extract is dissolved in pyridine, sodium nitroprusside solution and 20% NaOH is added to it. Formation of red color indicates the presence of cardiac glycoside.

Baljet test

To the extract sodium picrate solution was added. Formation of yellow to orange color indicates the presence of cardiac glycoside.

3.2 Test for Alkaloids

Mayers test:
3 ml of ethanolic extract was stirred with 3 ml of 1%HCl on steam bath. Mayers reagent (potassium iodide and mercuric chloride) was then added to mixture. Turbidity is formed due to the presence of alkaloid.
Dragendroff test:
To 2ml of the extract solution, Dragendroffs reagent (potassium bismuth iodide solution) was added. Orange brown precipitate is formed due to presence of alkaloid.
Wagners test:
To 2ml of the extract solution, Wagners reagent (iodine potassium iodide solution) is added. Reddish brown precipitate is observed due to presence of alkaloid.
Hager's test:
To 2ml of extract solution, Hager's reagent (saturated solution of picric acid) was added. Yellow precipitate is observed due to the presence of alkaloid.

3.3 Test for Tannins

Ferric chloride test
About 2ml of the ethanolic extract was stirred with 2ml of distilled water and few drops of FeCl3 solution were added. Formation of green precipitate indicates the presence of tannins.
Lead acetate test:
Ethanolic extract react with lead acetate (Pb (C2 H3 O2) 2) solution. Formation of white precipitate indicates the presence of tannins.
Gelatin test:
Ethanolic extract mixed with gelatin solution. Formation of cloudy precipitate indicates presence of tannins.
Potassium dichromate Test:
Ethanolic extract reacts with potassium dichromate. Formation of green color indicates presence of tannins.

3.4 Tests for Flavonoids

Shinoda test:
To 2ml of extract solution, few magnesium turnings, 5ml 95% ethanol and few drops of conc. HCl was added. Pink to red color develops due to the presence of flavonoids.
Sodium hydroxide test:
Add aqueous sodium hydroxide to the ethanoic extract. Yellow color develops that gradually disappears on addition of dilute acid (acetic acid) which indicate the presence of flavonoids.
Ferric chloride test:
To the extract add few drops of ferric chloride solution. A green colour indicates the presence of flavonoids

IV.PROCUREMENT OF MATERIALS FOR PROTEIN DENATURATION ASSAY

For the present study on anti-inflammatory activity using the protein denaturation method, egg albumin powder was employed as the protein source. The required chemicals were obtained from reliable commercial suppliers and were of analytical reagent grade. All experimental procedures were performed in the pharmacology laboratory of the Department of Pharmacology, Chemist College of Pharmaceutical Sciences and Research, Kerala, India, under controlled laboratory conditions.

4.1 Drug and Reagent

Egg albumin powder, Phosphate buffer (pH 6.4), Test extract (plant extract at different concentrations),
Standard anti-inflammatory drug (e.g., Diclofenac sodium), Distilled water

V.PROCUREMENT OF CHICKEN ILEUM

For the investigation, fresh chicken ileum was collected from slaughter house. It was kept at room temperature with adequate aeration in freshly prepared Tyrode solution. The research was done at chemist college of pharmaceutical sciences and research in Kerala, India, in the pharmacology laboratory of the department of pharmacology.

5.1 Drug and Chemical Reagents

Acetylcholine, Atropine, Ethanol

VI.EXPERIMENTAL DESIGN

6.1 Drug Preparation and Dilution (Protein Denaturation Assay)

The test extract stock solution was prepared by dissolving 100 mg of the extract in 100 ml of distilled water to obtain a concentration of 1 mg/ml. From this stock solution, further dilutions were made using distilled water to obtain the required concentrations for evaluation in the protein denaturation assay.
The standard anti-inflammatory drug (diclofenac sodium) was prepared following the same dilution procedure to obtain comparable concentrations. A fresh egg albumin solution was prepared by dissolving an appropriate amount of egg albumin powder in distilled water. Phosphate buffer (pH 6.4) was prepared separately and used to maintain suitable assay conditions throughout the experiment. All solutions were freshly prepared on the day of analysis to ensure reliability and reproducibility of the experimental results

6.2 Drug Preparation and Dilution of isolated chicken ileum

A concentration of $1x10^{-1}$ g/ml was created by dissolving 100mg of the extract in 100ml of distilled water, from this 10ml was taken and made up to 100ml with distilled water. The conventional medication, 100mg/ml of acetylcholine and atropine, were administered using the same process. Tyrode solution was prepared per litre of water by the dissolution of the following substances: Nacl- 8g, Kcl- 0.2g, $CaCl_2$- 0.2g, $NaHCO_3$- 1g, NaH_2PO_4- 1g, $MgCl_2$- 0.1g, Glucose- 2g.

6.3 Evaluation of Anti-Inflammatory activity by protein denaturation assay.

The anti-inflammatory activity of the test extract was evaluated using the protein denaturation method, based on the principle that prevention of heat-induced protein denaturation indicates potential anti-inflammatory properties. Egg albumin powder was used as the protein source and freshly dissolved in distilled water to prepare the protein solution. A reaction mixture was prepared by mixing 100 µl (0.1 ml) of the test extract at concentrations ranging from 100–500 µg/ml (0.1–0.5 mg/ml) with 200 µl (0.2 ml) of the egg albumin solution and 1400 µl (1.4 ml) of phosphate-buffered saline, pH 6.4. Distilled water was used instead of the extract for the negative control, while diclofenac sodium was used as the positive control.

The reaction mixtures were incubated at 37 °C for 15 minutes and then heated at 70 °C for 5 minutes in a water bath to induce protein denaturation. After cooling to room temperature under running tap water, the absorbance of each sample was measured at 277 nm using a UV–Visible spectrophotometer. All experiments were performed in triplicate, and the percentage inhibition of protein denaturation was calculated using the formula:

$$\% \text{ Inhibition of Protein Denaturation} = \frac{(C - D)}{C} \times 100$$

C = Absorbance of the control (without test sample)
D = Absorbance of the test sample (with extract or standard)

6.4 Evaluation of Spasmolytic Activity on Isolated Chicken Ileum.

Fresh chicken ileum was collected, placed in a beaker containing Tyrode solution at 37ºC, and then aerated. A portion of the ileum measuring 2-4 cm was excised, mounted, and kept at 37ºC in a 35-cc organ bath containing tyrode solution with oxygen supply. The kymograph and its attachments were set up so that the tissue would be properly tensioned. Before starting, the tissue was given 15 minutes to acclimatise.

Acetylcholine dose responses were established in the following order

1. Acetylcholine alone
2. Acetylcholine in presence of ethanolic extract of *Myristica fragrans Houtt*. leaves
3. Acetylcholine in presence of atropine

VII.RESULT

7.1 Phytochemical analysis

The chemical screening tests confirmed the presence of glycoside, alkaloids, flavonoids, and tannins in the ethanolic extract of *Myristica fragrans Houtt.* leaves

VIII.ASSESSMENT OF ANTI-INFLAMMATORYACTIVITY OF LEAVES EXTRACT

All experiments were performed in triplicate, and the data are presented as mean, confirming the consistency and reliability of the experimental results. The Leaf extract showed a concentration-dependent increase in the inhibition of protein denaturation, indicating improved protective activity at higher doses. The percentage inhibition ranges from 14.71% at 0.1 mg/mL to 25.53% at 0.25 mg/mL, and further to 30.88% at 0.5 mg/mL, demonstrating the ability of the leaf extract to stabilize proteins under heat-induced conditions. The standard drug, Diclofenac (100 µg/mL), produced 52.94% inhibition, which remained higher than that observed for the leaf extract at all tested concentrations. The low variability among replicate measurements reflects good experimental precision. Overall, the results indicate that the leaf extract possesses notable anti-inflammatory activity, though slightly lower than the reference standard, and supports its potential for further pharmacological evaluation.

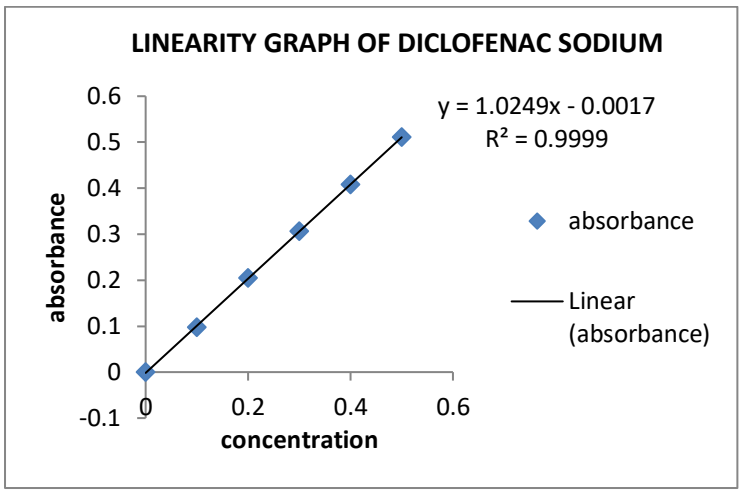

Figure 2: Linearity graph of diclofenac sodium

IX. ASSESSMENT OF SPASMOLYTIC ACTIVITY OF LEAVES EXTRACT

9.1 DRC of Acetylcholine alone

Dose of 0.1 ml, 0.2 ml, 0.4ml, 0.8ml was administered to the isolated chicken ileum and the DRC was recorded. The contraction produced in the smooth muscles of chicken ileum cause an increase in height of the response

9.2 DRC of Acetylcholine in presence of atropine

Dose of 0.1 ml, 0.2 ml, 0.4ml, 0.8ml was administered to the isolated chicken ileum by keeping the dose of Atropine (0.1ml) as constant, Atropine inhibits the activity of the Acetylcholine causes decrease in height of the response.

9.3 DRC of Acetylcholine in presence of ethanolic extract of *Myristica fragrans* leaves

Dose of 0.1ml, 0.2 ml, 0.4ml, 0.8ml was administered to the isolated chicken ileum by keeping the dose of Atropine (0.1ml) as constant, *Myristica fragrans Houtt.* leaves extract inhibits the activity of the acetylcholine which cause a decrease in height of the response

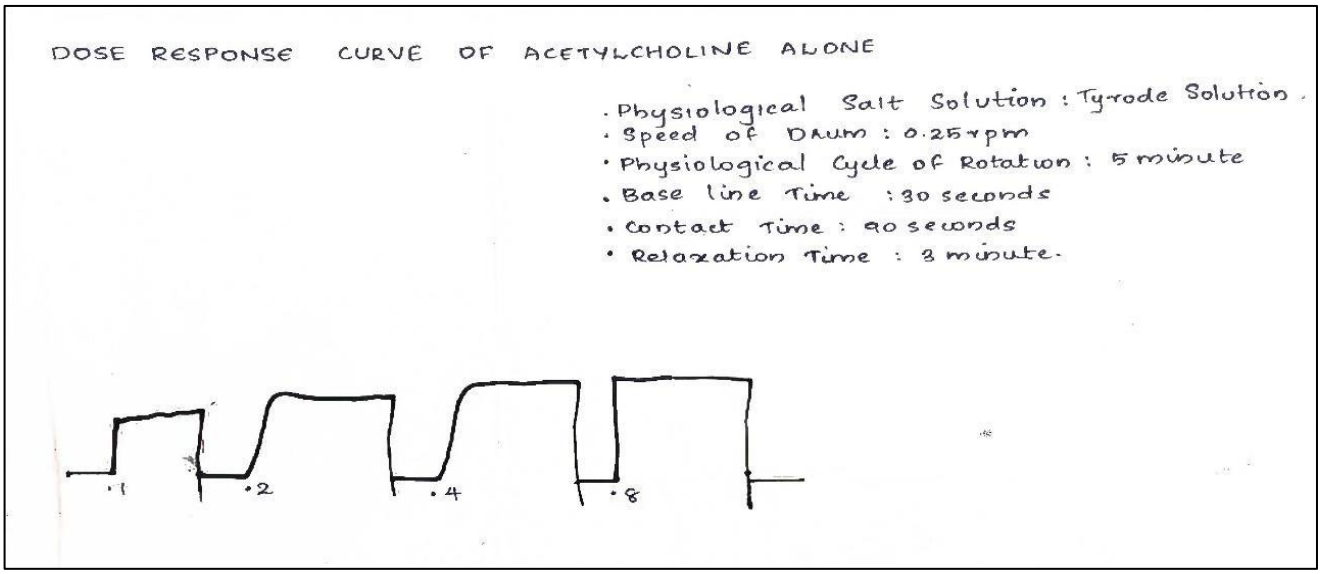

figure 3: dose response curve of acetylcholine alone

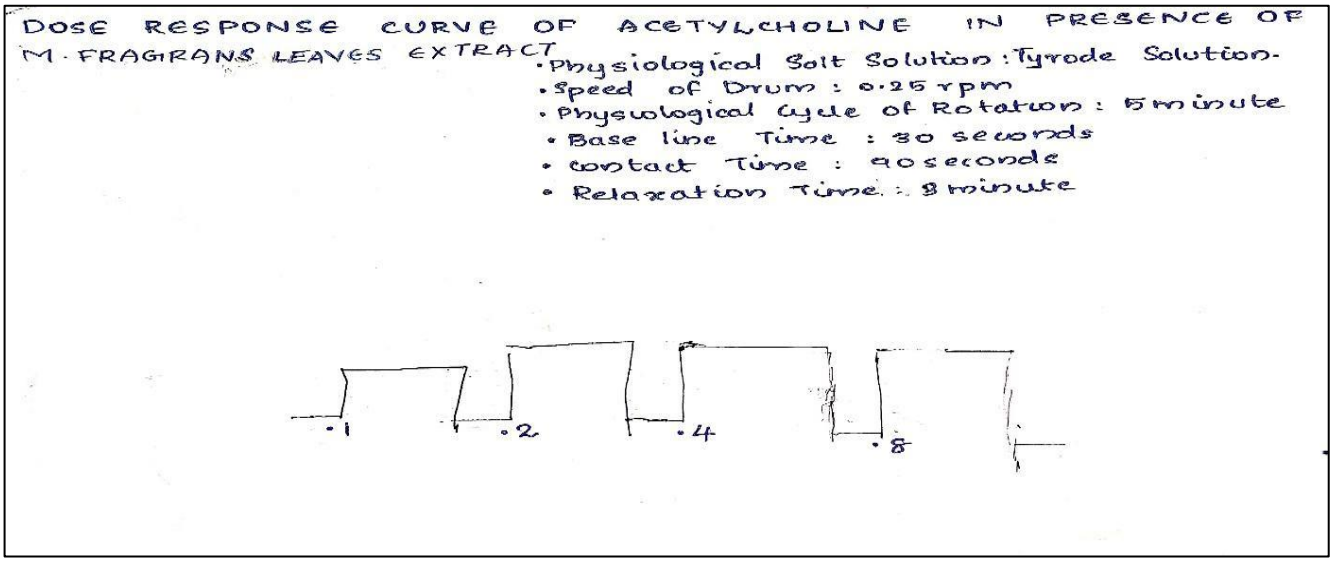

figure 4: dose response curve of acetylcholine in presence of *myristica fragrans houtt* leaves extract

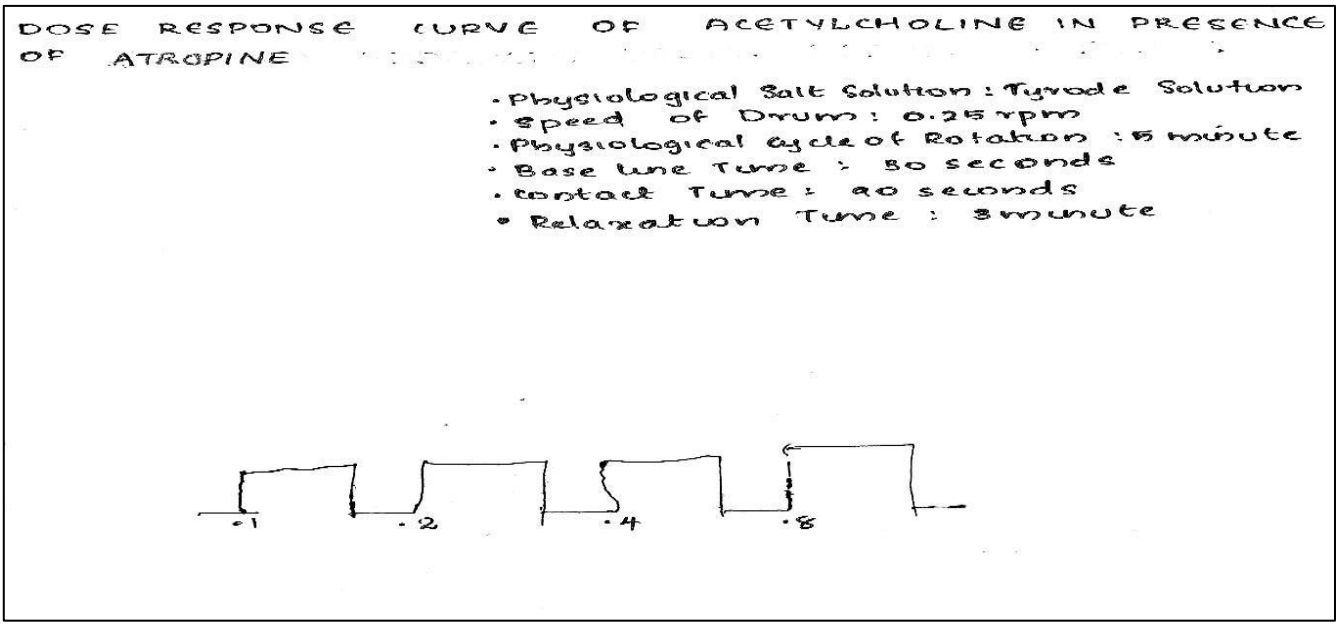

figure 5: dose response curve of acetylcholine in presence of atropine

Table:1 In- vitro response of an isolated chick ileum to acetylcholine alone and in the presence of leaves extract and atropine

SI NO:	Dose of ACH (ml)	Response of ACH alone		Response of acetylcholine in the presence of Atropine		Response of acetylcholine in the presence of leaves extract	
		Height in mm± SEM	% response	Height in mm± SEM	% response	Height in mm± SEM	% response
1	0.1	14.5±0.2887	63.88	8.3±0.8819	59.71	12.3±0.3512	57.7
2	0.2	18.7±0.3712	82.38	10.4±0.3055	74.82	17±0.5774	79.8
3	0.4	21.7±0.3512	95.59	11.3±0.8819	81.51	18.2±0.4163	85.44
4	0.8	22.7±0.2848	100	13.9±0.0882	100	21.3±0.881	100
Mean			85.46		79.01		80.73

X.DISCUSSION

The present study investigated the anti-inflammatory and antispasmodic potential of the ethanolic extract of *Myristica fragrans Houtt*. leaves. Anti-inflammatory activity was assessed using the protein denaturation assay, a widely used in vitro method for preliminary evaluation of anti-inflammatory effects. The leaf extract showed effective inhibition of protein denaturation in a concentration-dependent manner, indicating significant anti-inflammatory potential. The antispasmodic activity of the extract was evaluated using dose–response curve (DRC) analysis by comparing the responses of acetylcholine alone with those observed in the presence of atropine and the leaf extract. Acetylcholine induced pronounced smooth muscle contraction, whereas both atropine and the leaf extract significantly diminished the contractile response, indicating smooth muscle relaxation. The leaf extract may have a spasmolytic effect via interfering with calcium-dependent contraction pathways or muscarinic receptor function, as seen by the decrease in DRC height. Acetylcholine-induced smooth muscle contraction was significantly inhibited, as evidenced by the mean percentage responses of 85.46%, 80.73%, and 79.01% for acetylcholine, the leaf extract, and atropine, respectively. Overall, these findings indicate that *Myristica fragrans* leaves possess both anti-inflammatory and spasmolytic activities, which may be attributed to the presence of bioactive phytoconstituents such as flavonoids and phenylpropanoids. This dual pharmacological action highlights the therapeutic potential of the leaf extract in conditions associated with inflammation and smooth muscle spasms.

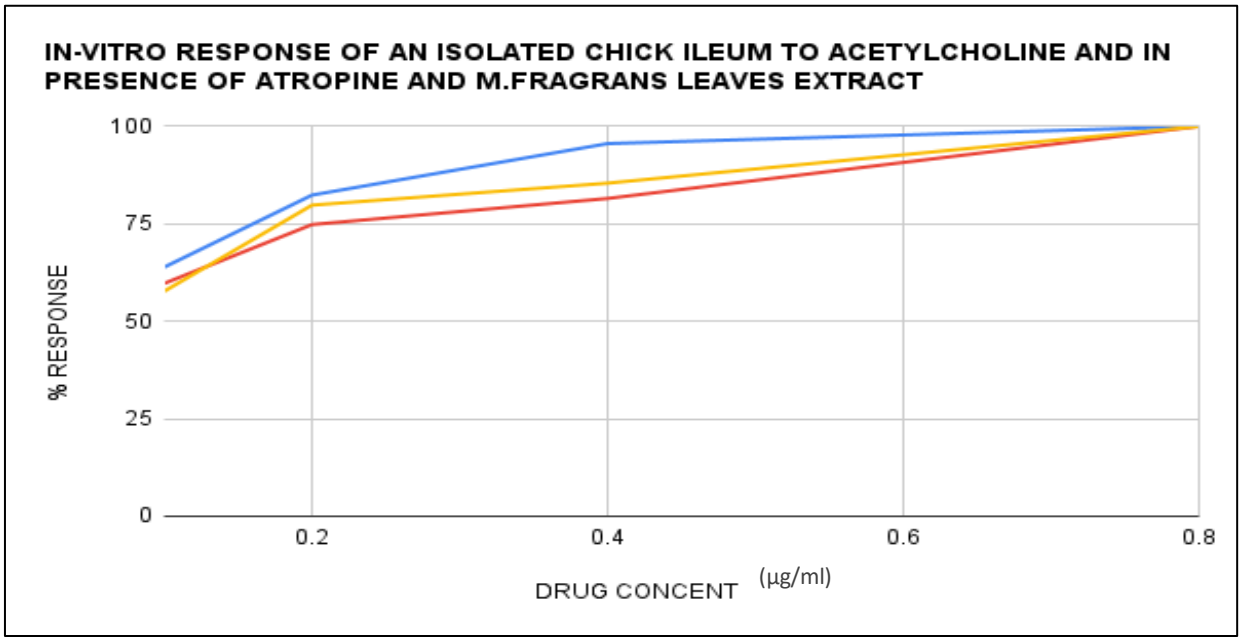

figure 6: in-vitro response of an isolated chick ileum to acetylcholine and in presence of atropine and *myristica fragrans houtt*. leaves extract

XI.CONCLUSION

The present study demonstrates that the ethanolic extract of *Myristica fragrans Houtt*. leaves exhibits significant anti-inflammatory and spasmolytic activities. The anti-inflammatory potential was confirmed using the protein denaturation assay, where the leaf extract effectively inhibited heat-induced protein denaturation in a concentration-dependent manner, indicating its ability to stabilize proteins and reduce inflammatory responses. This activity is likely mediated by bioactive phytoconstituents such as flavonoids and phenylpropanoids, which are known to possess anti-inflammatory properties.In addition, the leaf extract displayed notable spasmolytic activity in dose–response curve analysis of acetylcholine-induced smooth muscle contractions. The extract significantly reduced the contractile response, suggesting smooth muscle relaxation possibly through modulation of muscarinic receptors or calcium-mediated contraction pathways. The mean percentage responses confirmed that the leaf extract effectively attenuates acetylcholine-induced contractions, demonstrating its potential as a natural spasmolytic agent.Overall, these findings indicate that Myristica fragrans leaves possess dual pharmacological activity, making them a promising natural source for therapeutic applications in conditions involving inflammation and smooth muscle spasms, such as gastrointestinal disorders. Further studies, including in vivo evaluation and mechanistic investigations, are warranted to fully elucidate its therapeutic potential.

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