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RESEARCH ON TO STUDY ANTI-INFLAMMATORY POTENTIAL OF PEPPERMINT OIL USING IN-VITRO ANTI-INFLAMMATORY STUDIES BY PROTEIN DENATURATION METHOD

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ABSTRACT

Inflammation is a physical response to harm, illness, or injury that is marked through warmth, redness, tenderness, edema and aberrant physiological functions. Damage to connective tissue is often a part of the complicated network of connections among soluble substances and cellular components that make up the inflammatory process. Many diseases, such as allergies, autoimmune diseases, metabolic syndrome, cancer, and heart problems illnesses, are primarily brought on by an uncontrollably high inflammatory response, which has a substantial financial cost to both people and society at large. Common treatments for controlling and suppressing inflammatory crises include steroids, nonsteroid anti-inflammatory agents, and immunosuppressants. However, these drugs have a number of negative effects, most notably irritated intestines which can lead to gastric ulcers. Medical technology has advanced enormously as a result of the use of natural medicines.

Keywords*: Mentha piperita, Peppermint, Peppermint oil, Protein denaturation.*

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INTRODUCTION

Inflammation is a key component of various disease and natural compounds like essential oils have gained attention for their potential anti-inflammatory effects. Peppermint oil extracted from Mentha piperita is one such essential oil that shown promise in mitigation inflammation. Peppermint oil contains several bioactive compounds, notably menthol and menthone, which has been implicated in its anti-inflammatory activity. Despite its potential, the anti- inflammatory mechanisms of peppermint oil remain to be fully elucidated. Traditional anti-inflammatory drugs often come with adverse side effects, highlighting the need for safer and more effective alternatives. Essential oils derived from aromatic plants have been utilized for a long time in traditional prescription drugs for their therapeutic properties including anti-inflammatory effects. In vitro studies investigate the mechanisms of action of inflamed person compounds using cell cultures or isolated tissues. [(1)]

NSAIDs and other anti-inflammatory medications are used to lessen the pain and swelling associated with inflammation. However, extended use of these medications carries the risk of gastrointestinal toxicity, cardiovascular toxicity, and other toxicities. These reasons make it necessary to employ ant-inflammatory medications with fewer severe side effects for the treatment of chronic illnesses that cause inflammation. Consequently, previously, there an increase in curiosity about natural and alternative medications recently for the treatment of a variety of illnesses, although adequate scientific proof is lacking.

Peppermint, or Mentha Piperita, is a plant that is a member of the Lamiaceae family. Peppermint oil has been applied to several diseases, such as headaches, neuralgia, and colds, since ancient times. The antispasmodic qualities of peppermint oil are the main focus of this evaluation. Peppermint oil has a watery consistency, a clear to pale yellow color, and a strong, menthol-like fragrance. Menthol is the primary component of Dabur Pudina Hara, lozenges, toothpaste, pain and cold balms, and other products. The leaves of the Mentha plant are the primary ingredient in mint oil.

Ayurveda and other traditional Indian medicine have identified a number of plants on the Indian subcontinent as having anti-arthritic properties. Few of them have really been identified based on their mechanical properties, and these require further examination. Mentha Piperita is endemic to tropical and warm-temperate regions. It is also known by the names Pudina is a member of Lamiaceae. The plant is claimed to have beneficial properties as a tonic, diuretic, hepatoprotective, antioxidant, antibacterial, anti-inflammatory, and anti-arthritis agent. Many active compounds, such as flavonoids, tannins, menthone, vitamin C, vitaminA, glycosides, alkaloids and phenol have been discovered from the different plant portions. This study's objective is to evaluate the effectiveness of Metha Piperita whole plant extract treating the inflammation. [(2)]

To assess in vitro anti-inflammatory effects, membrane stabilization assays and protein denaturation assays are often utilized. A minimum of three in vitro tests have been employed by numerous studies to assess the anti-inflammatory properties of herbal components. The medications that are most commonly used as reference standards include indomethacin, acetyl salicylic acid, and diclofenac. Since the wellbeing of both humans and animals is crucial, we recommend reducing the use of animals in anti-inflammatory activities in vitro investigations [(3)]

Inflammation, an actual response to damage, injury, is typified by warmth, redness, soreness, edema, and abnormalities in biochemical functions. [(4)] When physical trauma, hazardous chemicals, or microbial infections damage tissues, inflammation is often the body's defensive reaction. The physique's reaction is to eliminate the aggravating factors, deplete the alien species, and prepare the tissue for repair. [(5)]

Irritation can be classified into two categories: acute and chronic.

Increased blood flow of leukocytes and plasma into wounded tissues is the source of acute inflammation, the body's first line of defense against harmful stimuli. The acute inflammatory process is started by cells that are already present in the tissues. The two primary vascular changes that characterize this are vasodilatation and enhanced capillary permeability, both of which are brought on by different inflammatory mediators. [6]

PRINCIPLE:

In vitro Egg Albumin Denaturation Method:

The experiment utilizes egg albumin as a model protein, which is denaturized by subjecting it to high temperatures, high pH values, or other denaturing chemicals. During denaturation, egg albumin's initial conformation ruptures, altering its physical properties and rendering it non-functional. [7] Consequently, compounds or agents with anti-inflammatory qualities may be those that dramatically lower the denaturation of egg albumin in this experiment. [8-9]

In vitro protein denaturation is the process of altering a proteins natural structure and function in a controlled laboratory environment. [10]

Protein denaturation has been proposed as one of the causes of inflammation. As they suppress the COX enzyme, NSAIDs additionally prevent denaturation of proteins. In order to determine % inhibition, it is possible to incubate test samples at different concentrations using egg albumin solution. under carefully regulated circumstances for the experiment. These will enable the responses to take place & determine absorbance. By using GraphPad Prism system, IC50 values can be calculated. As a reference drug being used, diclofenac sodium. [11]

PLANT PROFILE:

Fig. No.1: Mentha piperita plant

METHODOLOGY:

Literature review

Selection, collection, and authentication of plant
Drying, sifling and Extraction of the Whole plant material.

Phytochemical investigation & TLC
In vitro study

Protein Denaturation method

Results and Discussion

Conclusion

PROCEDURE:

Plant sample pre-extraction preparation for soxhlet extraction:

Plant samples are created before being extracted, phytoconstituents in the plants are preserved, which is initial stage in the research of medicinal plants. The extraction process then makes use of plant elements such leaves, stems, barks, roots, fruit, and flowers. Before the extraction, the ensuing requirements are crucial. [12]

Selection and collection of plant materials:

Effective phyto component isolation depends on careful plant material selection and collection. Only healthy, disease-free for plant extraction, plants that are protected from weeds and insects are preferred. as well as many variables involved in gathering the plant materials. The recommendations for the collection of plant material, including the collection of seed and vegetative collections, were developed by the Plant Materials Program of the NRCS. This guideline describes the appropriate time in light of collecting, how to gather it, how to process it, and how to store it. [13]

Drying of plant materials:

In order to extract plant materials, the drying process is necessary. Since fresh plant materials contain active enzymes that produce active constituents, intermediates, and metabolic reactions, drying is also necessary for the preparation of plant materials prior to extraction. [14] Since heat can cause volatile compounds from plant materials to be lost and light can cause some light-sensitive constituents to be lost, many researchers are using the air-dry method in a shaded, dark area to dry their plants.

Additionally, plant materials can be dried using some of the dry techniques such freeze drying, silica gel or salt drying, oven drying, and microwave drying. [15]

Reduced size and grinding:

Reduced size and grinding are necessary for soxhlet extraction process because smaller particles have a larger surface area when they are powdered. large surface area improves the powdered particles' interaction with the solvent of extraction, leading to a more successful extraction. To reduce size, a variety of milling and grinding techniques are used like cutter mills, plate mills, roller mills, hammer mills, and fixed head mills. [16]

Fig. No. 2: Powder of Mentha piperita

Size separation/sieving:

For effective soxhlet extraction, size separation is crucial. Because the solvent may flow through the powdered particles placed in the thimble consistently, uniform powdered particle size maximizes extraction. It is not possible to extract effectively using very fine or very coarse particles. When extracting, very fine powders can create beds, and very coarse powders can slow down the procedure for extraction. The difference in size process is carried out using the size of the particles and the method of sifting is determined using various techniques such as microscopic analysis and sieving. [17]

Choosing a solvent for the soxhlet extraction process:

Depend on the procedure of isolating the phyto constituents, the solvent for soxhlet extraction is chosen. It need to be simple to take out and neutralize the solvent. The rising polarity order of solvents, such as water, methanol, ethanol, acetone, petroleum ether, ethyl acetate, and chloroform are typically accustomed to determine which solvent to use. Steroids and fixed oils are frequently extracted using petroleum ether, which is also used to remove chlorophyll from leaf powder. [18] To defatten the plant material, some researchers employ petroleum ether. Following defatting, the primary solvent was extracted using either alcohol or water. Since waxy materials hinder the extraction process and cause the solvent to emulsify more quickly. defatting some plant components is necessary. [19] Water is a polar solvent that is harmless and inexpensive, while methanol is a semi-polar solvent that can extract several phytoconstituents. Water separates a large number of polar components, making them ideal for research on both humans and animals. [20]

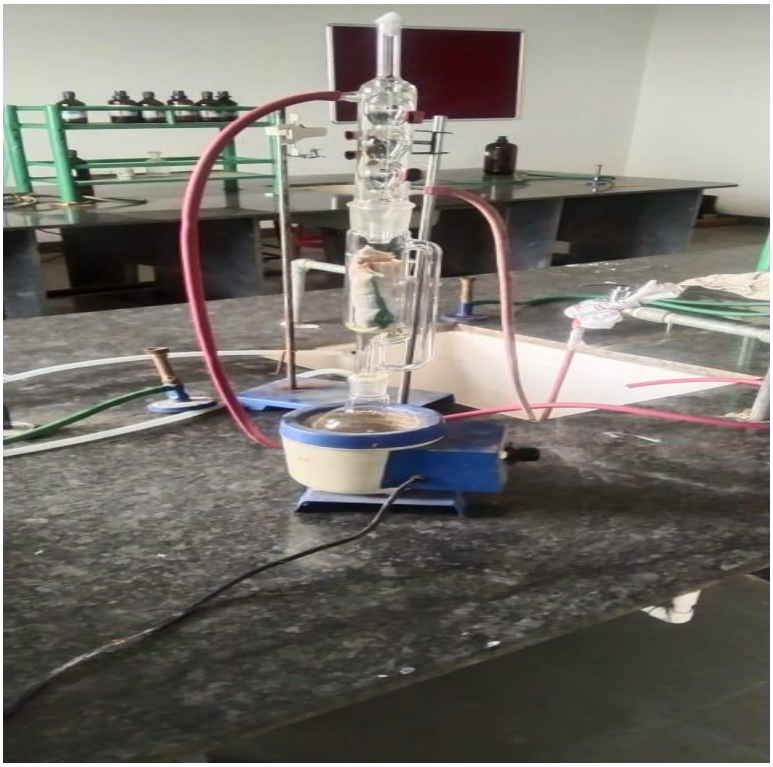

Fig. No.3: Soxhlet Apparatus

Post extraction process:

Following the soxhlet extraction procedure, the extracted components can go through the following steps: extract concentration, solvent evaporation, and extract storage. The extraction of extract from solvent is accomplished by the use of different evaporators or the distillation process. [21] Numerous researchers employ distillation techniques, rotary evaporators, and standard air-drying procedures to produce concentrate extracts. Once the extract has been collected, it is kept refrigerated in a tightly sealed container wrapped with aluminium foil. [22-24]

Fig No.4: Extract of Mentha piperita

Protein Denaturation by Using an Egg Albumin:

PROCEDURE:

Control- 2ml distilled water,2.8ml PBS (pH 6.4), 0.2ml fresh egg albumin.

Standard- 2ml Diclofenac Sodium as (50 µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml, 1000 µg/ml)

Test - 2ml different con. of MEPT

Blank- 2.8ml PBS and 2.2ml Distilled water

Incubated for 15min at 37℃ heated at 70℃ for 5min absorbance at 660nm on a UV Visible spectrophotometer.

Fig No.5: Different Concentration in test tube for testing

RESULTS

The control group had an absorbance value of 0.896. Diclofenac sodium (the standard) showed a concentration-dependent increase in % inhibition, ranging from 34.06% to 68.04% at concentrations from 100 to 1000 µg/mL. *Mentha piperita* extract also showed a concentration-dependent increase in % inhibition, ranging from 7.21% to 21.66% at concentrations from 100 to 1000 µg/mL. Diclofenac sodium, a known anti-inflammatory drug, exhibited significant anti-inflammatory activity in a dose-dependent manner. Mentha piperita extract also showed anti-inflammatory activity, although to a lesser extent compared to diclofenac sodium. The study indicates that *Mentha piperita* may possess anti-inflammatory properties.

Sr. No	Description	Concentration (microgram/ml)	Absorbance	%Inhibition
			Mean	
1.	Control		0.896	
2.	Standard Diclofenac Sodium	100	0.764	34.06
		200	0.623	46.30
		400	0.583	50.46
		800	0.421	56.67
		1000	0.315	68.04
3.	*Mentha piperita* Methanolic extract	100	0.826	7.21
		200	0.781	12.36
		400	0.624	22.61
		800	0.546	38.47
		1000	0.435	55.68

Table No.1: Result of Testing Sample

CONCLUSION

One of the most well-known causes of inflammation is tissue protein denaturation. A number of intricate processes, including membrane rearrangement, increased protein denaturation, and increased vascular permeability, take place when there is inflammation, which is usually uncomfortable. Studying & analysing natural vegetation may result in identification that of brand-new bioavailable substances with significant anti-inflammatory properties. The natural flora's inspiration has been essential in the creation of novel treatments.

Thus, in order to conduct analysis in order scientists need simple, basic methods to collect additional data and evaluate the efficacy of natural anti-inflammatory medications. Inexpensive in vitro procedures. In this instance, inflammation is believed to be caused in part by denaturing of proteins. To enable reactions of various test sample concentrations can be incubated in a regulated environment using egg albumin solution. laboratory environment. The percentage of inhibition can then be ascertained by measuring the absorbance. GraphPad Prism programme can then be used to calculate IC50 values. Diclofenac sodium has several uses model drug, and researchers can draw conclusions about their investigations by applying statistical analysis techniques.

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