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MEMECYLALENE NANOEMULSION PROPERTIES AGAINST INFLAMMATION AND ANGIOGENESIS.

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ABSTRACT

Nanoemulsions of Memecylaene were formulated and evaluated for their anti-inflammatory, antioxidant, and antiangiogenic properties. Several chromatographic methods were used to isolate memecylaene from Memecylon malabaricum leaves.Sunflower oil served as the oily phase, Tween 85 served as the surfactants, and C2H5OH served as the co-surfactant in the sonication process used to create memecylaenenano-emulsions. A zeta potential experiment and droplet size analysis were performed on the nanoemulsion. Studies of stability were carried out, as well as biochemical tests on the nanoemulsions. A zeta potential of - 1.30 mV was determined for the synthesized nanoemulsion with particle sizes ranging from 60.05 nm to 60.50 nm. Embedded Memecylaene in O/W emulsions exhibits enhanced anti-inflammatory and anti-secretagogues effects by inhibiting phospholipase (PLA2) enzymes and H +, K+-ATPases, therefore demonstrating enhanced activity. Nitric oxide radicals scavenging activity and DPPH radical scavenging activity were used to evaluate anti-oxidant activity in vitro. Angiogenesis is also inhibited in chick chorioallantoic membrane (CAM) assay, which is an in-vivo model system that is dependent on angiogenesis. Memecylaene-loaded nano-emulsions have the potential to be used as therapeutic agents based on the findings above

KEY WORDS: Anti-oxidant and -angiogenesis, bioactive compounds, Memecylaene, Nanoemulsion, chick chorioallantoic membrane, Phospholipase A2 (PLA2).

INTRODUCTION

This plant originated in India and is known as Memecylon malabaricum. This includes several more bio-active substances in combination to steroids, triterpenes, flavonoids, saponins, tannins, and resins [1, 2]. There were many skin problems that can be cured with the leaves, including chicken pox, polyuria, menorrhagia, and herpes [3]. A study conducted in vivo and ex vivo found memecylaene to inhibit angiogenic, antiproliferative, proapoptotic, antioxidant, and anti-inflammatory activity. It possesses an unusual structure that mimics crown ethers and has a promising biological potential [4]. As a highly lipophilic compound, memecylaene has low absorption and dissolution problems, which can reduce its efficacy. It is therefore essential to improve Memecylaene's solubilisation property and modify its pharmacokinetic profile through a prospective drug delivery mechanism [5]. To alter its physicochemical characteristics, a new formulation is needed.

Due to its greater reactivity in sunflower oil, the molecule is easily accessible for transdermal administration [6]. Without using high-energy techniques, a straightforward nano-emulsion (NE) system was devised.In unstable NE formulations or by their hydrophobicity, NE has always posed a major challenge in drug formulations [7, 8]. To improve hydrophobic drug solubility and bioavailability, nanoparticles (NPs) are being used as drug delivery systems. A nanosized droplet would increase the interfacial areas associated with NPs, which would impact the drug's transport properties [9].

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Due to their segmented hydrophobic domains, NEs can include both polar and non-polar substances [10]. Numerous studies on NE systems produced from plant-based essential oils that might be used in medications have been reported [11]. The goal of this work was to increase the efficacy and reliability of an oil-in-water NE delivery device for memecylaene without resorting to high energy techniques [12]. Consequently, the system should be able to transport easily through the smallest capillaries without being discriminated by the host's defense mechanism [13].

METHOD AND MATERIALS

Information on Materials:

Sodium Hydroxide buffer, Bragg's ringer buffer, 2 mM HEPES-Tris, Sucrose-EDTA buffer, butylated hydroxyl toluene (BHT), 1, 1-diphenyl 2-picrylhydrazyl (DPPH), and Sunflower oil were used for the analysis of time-lapse images.

Techniques

Memecylaene Isolation:

An Indian taxonomist identified and authenticated the leaves of *Memecylon malabaricum*. Drying and grinding were done in the shade. As a solvent system, hexane was used to extract the ground leaves with the Soxhlet. We loaded the extracted sample onto a column filled with silica gel (150-250 mesh) and evaded the sample in CHCL3 after filtering. Precipitated fractions were filtered and precipitated using methanol. A thin layer chromatography method was used to purify memecylaene from residue. A nano-0.0001kg emulsion was prepared using the Memecylaene obtained thus far.

A Method for Preparing Nanoemulsions:

Sonication was used to prepare NE. A beaker placed on a magnetic stirrer was filled with 0.002l of Smix (2:2.5 ratio of Tween 85: C_2H_5OH) dissolved in 0.001kg of Memecylaene. Drops of water were added over a specified period of time, and then the liquid was swirled on a magnetic stirrer to ensure that it reached a consistent consistency. The complete combination was sonicated with probes after just a predetermined period of time and speed. The generated NE's formulations table is shown in Table 1.

A Study of the Stability of Nanoemulsions:

The composition was kept at every temperature for a minimum of two days throughout each of the 4 cycles, and its consistency was assessed at each temperature. A phase separation test was conducted on the formulation by centrifuging it for 35 minutes at 4000 rpm. Observations of phase separation were made after 4 freeze-thaw cycles between -25°C and +30°C were performed on the formulation.

Analyzing the Nanoemulsion's Properties:

Measurement of Surface Charge, Poly dispersity Index and Particle Size Analysis:

Malvern zetasizers were employed to examine the distribution of particle sizes and size of NE preparations. Size of the particles, size distribution, and zeta potential were all determined using DLS.The peaks were higher before filtration and lower after filtration. The Malvern zetasizer was used to measure the zeta potentials (ζ) of the NE formulations.

Characteristics of the Visual Appearance:

In addition to examining its texture, the NE was examined for its visible structures.

The Indirect Hemolytic Method for Phospholipase A2 (PLA2) Is As Follows:

In accordance with the Boman HG and Kaletta8 approach, adult erythrocytes, egg yolk, and phosphate - buffered saline saline (2:2:7 V/V) were combined to test the PLA2 activities. Phosphate buffered saline (2:2:7 V/V), egg yolk, and packed human erythrocytes were combined in the correct proportions. Alternatively, this mixture was incubated with 65 g of *V.russelli* poison for 10 minutes at 38°C, and then centrifuged at 0.8 kg for 15 minutes at 5°C with the addition of 0.01 l of ice-cold phosphate buffer saline. The frequency at which haemoglobin in the supernatant is detected is 545 nm.A total of 3, 6, 9, 12, and 15 µg of NE were used in the assay. An experiment in which distilled water was added to the control reaction mixture resulted in 99.9% lysis of erythrocytes.

Activities of Gastric H^+/K^+-ATPases:

An $Mg2^+$-dependent ATPase activity of 1.5 mmol/l was measured in 1.5 ml of 1 mmol/l ATP and 55 mmol/l Tris-HCl buffers (pH 7.6) stimulated by K^+ and HCO_3. A basic medium sample was used to determine ATPase activity. After adding substrate (ATP), the reaction was carried out at 38^OC for 20 minutes, and the liberated inorganic phosphate from ATP was measured by Tsai method using 1.5 ml of ice-cold 25% tri chloroacetic acid (TCA).

Three independent experiments were performed in triplicate. NE concentrations of 3, 9 and 15 µg were also tested. A comparison between NE's inhibition and that of the drug Omeprazole (Ome) was made to calculate the percentage inhibition. On the basis of the concentration-inhibition response curve, 55% inhibition was calculated (IC55 µg/ml).

RESEARCHONANTIOXIDANTS

Scavenging Of Radicals by DPPH:

This study was conducted in accordance with Lingappa Mallesha *et al*. briefly; different aliquots of NE were mixed under vigorous shaking together with 0.001 liters of DPPH solution (0.5 mM in 96% ethanol). An

absorbance test at 520 nm was conducted with a UV-VIS spectrophotometer (HITACHI) after 25 minutes of standing at room temperature. This is expressed as an IC55 value, which means the concentration at which 55% of DPPH radicals can be scavenged. DPPH radical scavenging assays were performed using BHT as a positive control [14].

Radical Scavenging By Nitric Oxide:

Nitric oxide was produced by the Griess reaction employing sodium nitroprusside. The introduction of sodium nitro prusside at physiological pH12 results in the generation of NO (nitricoxide). NO and O_2 produce nitrate as a product of their reaction, which is estimated using Griess reagent. With slight modifications, Lingappa Mallesha *et al* examined the radical scavenging activity of nitric oxide. Competition between nitric oxide scavengers and oxygen reduces nitric oxide production. NE was diluted in phosphate buffered saline (3, 6, 9, 12, and 15 g) and mixed with sodium nitro prusside (6mM) and then incubated for 180 minutes at 30°C. Using the Griess reagent at 550 nm, the absorbance of sulphanilamide with nitrite was used against BHT which was treated in a similar way [15].

Assay of Chanoallantoic Membrane (CAM) Angioinhibition:

Angioinhibitory activity of a compound can be validated using shell-less chorioallantoic membrane assay. To investigate the antiangiogenic activity of Memecylaene, the CAM assay was performed. A slightly modified version of Bushra Begum's method was used to examine the antiangiogenic effect [17]. An alcohol solution of 75% was used to surface sterilize fertilized hen's eggs. Eggs were incubated at 38°C in a fan-assisted humidified incubator. Eggs that were five days old were cracked out of a hammock and placed in a laminar air flow for incubation. It was observed that the eggs inside the hammock exhibited proliferating blood vessels on day 4; later, the filter paper discs containing 6 g of Memecylaene NE were placed over these proliferating blood vessels and the eggs were incubated again. One-day incubation resulted in an antiangiogenic effect for the molecule [16].

Table1. **Flow chart for formulations:**

PREPARATION	OIL	SURFACTANT-MIX [TWEEN 85+C_2H_5OH]	H_2O	CO-SURFACTANT (TWEEN 85: C_2H_5OH)	OIL: SURFACTANT MIX
Memecylaene NE	0.0005g	0.002l	0.0075l	2:2.5	2:5

A study of the stability of nanoemulsions:

Phase transition was not seen in the generated NE following the heating-cooling cycle, centrifugation test, or freeze-thaw cycle. Because there were no deposits, as the NE was stable.

The nanoemulsion was characterized as follows:

The size of the nanoparticles of the drug-loaded NE was determined to lie in the range of 60.05nm to 60.50nm, while those of the blank NE was determined to be between 11.58nm and 11.65nm. Drug-loaded NE had a PDI of 0.990 compared to blank NE with 0.450. NE blanks had a zeta potential of -1.25 mV and NE loaded with drugs had a zeta potential of -1.30 mV.

Characterization by visuals:

After two months of observation, the formulation retained its visual appeal and homogeneity. An NE formulation meets the NE criteria by being transparent pale yellow in appearance.

Analyzing phospholipase A2 via indirect hemolysis:

The IC55 value of NE was 9.95g/ml, which indicated dose-dependent inhibition of Russell viper venom PLA2. Inflammation inhibition was caused by PLA2 enzymes increasing with NE concentration.

Inhibition of Gastric ATPase (H^+K^+):

Fundic areas of sheep were collected and stored in sucrose EDTA buffer. Different gastric membranes were tested for ATPase activity. Tsai method was used to estimate inorganic phosphate liberated from ATP. NE inhibits (H^+ - K^+) ATPase more strongly than Omeprazole, the standard drug. As a result, there was a dose-dependent inhibition of ATPase. Comparing NE with omeprazole, the reference drug's IC55 was 9.35g/ml, while NE's was 7.60g/ml.

STUDIES ON ANTIOXIDANTS:

Scavenging Of Radicals by DPPH:

Scavenging free radicals is one of the characteristics of natural antioxidants. Proton radical scavenging by antioxidants can be measured using the DPPH scavenging assay. Its maximum absorbance is 520 nm. Antioxidants scavenge proton-radicals, which results in a decrease in absorbance maxima. A DPPH molecule donates hydrogen to free radicals, demonstrating its ability to scavenge free radicals. As a hydrogen donor, NE scavenges DPPH radicals. A Memecylaene loaded NE was shown to be capable of scavenging DPPH radicals with an IC55 value of 6.20µg/ml, which is comparable to the IC55 value of 04.40 µg/ml for BHT. As the concentration of NE increased, a higher amount of radical scavenging activity

was observed in comparison to BHT, the reference compound.

The Scavenging Of Radicals by NO (Nitric-Oxide):

At the physiological pH, NE of Memecylaene inhibited nitric oxide generation by sodium nitro prusside by an IC55 value of 4.7 µg/ml and NE of BHT by a value of 4.85 µg/ml. NE and BHT scavenge nitric oxide radicals almost equally when tested in the same study.

CAM-Based Assay of Angiogenic Inhibition:

Shell-less CAM assay model of developing embryos showed significant evidence of the angio-inhibitory activity of NE. Based on a least of seven eggs per group, the results are represented in the data shown. Memecylaene'santiangiogenic activity was investigated with discs loaded with 6µg of NE and demonstrated significant reduction in proliferation of capillaries. NE produces a powerful antiangiogenic effect in vivo, indicating its potent antiangiogenic properties.

This plant material contains a number of bioactive compounds, such as steroid, triterpene, flavonoid, saponin and tannin compounds. The methanolic extract of this plant exhibits antimicrobial and diabetic properties. Due to the hydrophobic characteristic of the memecylaene compound, a NE was created to improve its solubility and assess its physiological features. All of these investigations indicate that NE can improve Memecylaene's solubility, which is achieved by mixing alcohol and Tween 85 with oil and water to dissolve it. Memecylaene, a hydrophobic molecule, and co-surfactant (S_{mix}), which enclose it, make up nano-particles.

CONCLUSION

Memecylon malabaricum produced a hydrophobic compound that was separated and shown to have strong metabolic processes, and is therefore suitable for use as an easily accessible drug for transdermal administration. Stable drug-loaded NE was synthesized by properly selecting all the critical components. As of now, NE has been shown to be capable of protecting labile drugs, controlling their release, increasing the bioavailability of the drugs, and reducing patient variability and increasing their solubility**.** In accordance with the findings of these experiments, NE of the O/W type may increase the solubility of the hydrophobic molecule memecylaene.

Memecylaene's capacity to lower pro-inflammatory indicators and boost antioxidant property through triggering PLA2 inhibition is evidence of its anti-inflammatory potential. Our research demonstrates that NE of Memecylaene, sunflower oil, Tween 85, and water may be utilised to successfully dissolves Memecylaene, in which is poorly water-soluble. Memecylaene can dissolve in sunflower oil up to 15 mg/ml. When introduced into the oil core of the NE system, it also retained strong thermodynamic stability and a greater drug-loading potentiality. As a result of particle size reduction to nanometre range, drug dissolution rate would improve and bioavailability would be improved *in vivo.* This will enhance the therapeutic efficacy of the drug. In addition, pharmaceutical scientists will find it interesting to develop NE formulations of Memecylaene that involve no high-energy methods and which are optically clear and low-viscosity.

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