CREATION AND IN-VIVO EVALUATION OF THE POLYHERBAL NANOEMULGEL FOR THE TREATMENT OF INFLAMMATION

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Abstract:
Nyctanthes arbor-tristis is an Ayurvedic herb that has been used for centuries in India for its medicinal properties. The herb has anti-inflammatory, analgesic, and antipyretic qualities, this plant is well-known in traditional Indian medicine, specifically in Ayurveda, for its diverse pharmacological properties. It is utilized for its antispasmodic, anti-arthritic, anti-inflammatory, antidiabetic, immune-stimulant, antibacterial, antileishmanial, antipyretic, antidiabetic, antiviral, antimicrobial, antioxidant, anthelminthic, anti-allergic, hepatoprotective, and CNS depressant actions.
Nano-emulsiongel: The nanoemulgel's improved dermis-layer adhesion properties and increased solubility provide a raised concentration gradient in the epidermis, which affects the amount of medicine that diffuses into the epidermis as it descends the gradient. This nanoemulgel has been the attention of several researchers who are working to develop a variety of drugs that can be utilised to treat different dermis problems.
Result: At 1, 2, 3, 4, and 5 hours after receiving a carrageenan injection, the group treated with the high dose of Polyherbal Nanoemulgel showed a significantly lower level of paw edoema than the reference control group (p0.05). Although the paw edoema was reduced in the low and mid-dose groups as well, the difference from the reference control group was not statistically significant.
Conclusion: The findings of this study show that, in comparison to the normal control group, the polyherbal nanoemulgel dramatically decreased the paw edoema volume in a dose-dependent way. The greatest anti-inflammatory efficacy was seen at the high dose of the nanoemulgel (500mg).

Keywords: *Nyctanthes arbor-tristis*, Nano-emulsion gel, anti-inflammatory, In vivo study

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1. INTRODUCTION

The plant-based medicines which are being used for the treatment of various diseases are practiced since the ancient time. There are various methods including Ayurveda, Unani, and Siddha medicines are being used [1]. The constituents of the plants are the most desirable discovery and validation in several medication therapies.

Plant based medicine novel and functionally active in terms of diversify pharmacological activities of several classes of phytochemicals. These bioactive molecules are referred as secondary metabolites sometimes include flavonoids, alkaloids, terpenoids, and phenolic compounds [2]. These phytochemicals categories are often classified as pharmacological lineup for the treatments of several severe diseases by lowering the risk of diseases and promoting the healthy Wellness. Is it is validated that these plant extracts possess numerous biochemical properties such as anti-inflammatory, Antidiabetic, anti-fungal, antimicrobial and anticancer [3]. These plants are potentially active to get attention in various fields of the pharmaceutical industries such as food, cosmetic and therapeutic [4]. Inflammation is the body's response to infection, irritation or injury. The main symptoms of inflammation are redness, swelling, pain and heat. Inflammation is a natural action that assists the body to cure and safeguard itself from distress. Sometimes inflammation can become chronic and lead to health problems [5]. There are a number of different reasons that inflammation may occur. One reason is due to an injury or infection. When the body is injured, it releases chemicals that cause blood vessels to swell and tissues to become warm and tender [6]. This helps to protect the area and promote healing. However, sometimes inflammation can occur without an apparent injury or infection, which is known as chronic inflammation. This type of inflammation is thought to be caused by a number of factors, including genetics, diet, stress, and environmental exposure [7]. Common etiology of inflammation is infection, trauma, or autoimmune disease. Infection-related inflammation is most often caused by viruses, bacteria, and other microorganisms that invade the body [8]. Trauma-related inflammation is usually the result of tissue damage from a physical injury or exposure to a chemical irritant. Autoimmune disease-related inflammation occurs when the body's immune system attacks its own tissues [9]. *Nyctanthes arbor-tristis* is used for the colds, coughs, and fever. Aloe Vera is used for the burns, wounds, and skin diseases [8].

The traditional use of *Nyctanthes arbor-tristis* and *Aloe Vera* in inflammation and cancer is well documented. Both herbs have been effective in various inflammatory conditions, including arthritis, cancer, and colitis [10]. The anti-inflammatory and anticancer effects of Nyctanthes arbor-tristis and Aloe Vera have been demonstrated in a number of studies. These plants contain compounds that can modulate the activity of inflammatory mediators, such as cytokines and eicosanoids. In addition, they also possess antioxidant properties that can scavenge reactive oxygen species and protect cells from oxidative stress [11].

Nyctanthes arbor-tristis and *Aloe Vera* are two of the most potoar plants in the world. They both have a wide range of benefits and uses that make them essential to many people [12]. *Nyctanthes arbor-tristis* is a flowering tree and is native to India and Pakistan. It is also known as the night-flowering jasmine or Parijat tree. Tree can reach a height of up to 20 feet tall. It bears small, white flowers that bloom specifically during night [13]. Aloe Vera is a succulent plant that is native to Africa, Arabia, and Madagascar. It has thick, fleshy leaves that are filled with a clear gel. This gel has many healing properties and is often used in traditional medicine [14]. *The Nyctanthes Arbor tristis* is an evergreen tree. The tree grows to a height of 5-15m and has fragrant white flowers that bloom in the evening [15]. The tree is also known as the night-flowering jasmine or the Parijata tree. They are native to tropical and subtropical regions of Asia, including countries such as India, Indonesia, Malaysia, Philippines, Sri Lanka, and Vietnam [16]. The *Nyctanthes arbor-tristis* is also known as the "Tree of Sorrow" because its flowers bloom at night and fall off by morning, symbolizing the transitory nature of life [17,18,19]. The *Nyctanthes Arbor tristis* has many medicinal properties and is used in Ayurvedic and Chinese medicine. Unani and Ayurveda medicines are safe and effective, with minimal side effects. They are easily available and affordable, making them accessible to all. These factors make traditional systems of medicine an attractive option for healthcare. The leaves of the tree are used in fevers, diarrhea, and dysentery and bark is used in skin diseases [20,21,22]. The leaves and flowers of *Nyctanthes Arbor tristis* are rich in alkaloids, flavonoids, terpenoids, and saponins, which are known to have anti-inflammatory activity. TheClinical studies examining the effect of polyphenol-rich foods on inflammation have generally shown positive results, although more research is needed in this area [23,24].

Conventional Treatment And Their Limitation

A conventional drug delivery system is the method of treatment for the delivery of drug into the body. The examples are-Oral Delivery, Sublingual Delivery, Intravenous Delivery, Subcutaneous Delivery and Intramuscular Delivery

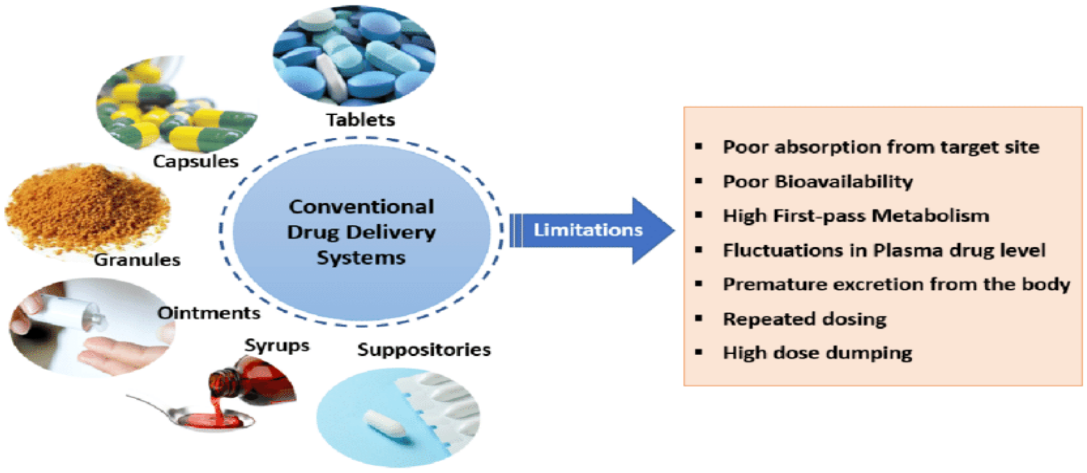


Fig.1: Limitations of conventional dosage formulation

2. MATERIAL AND METHODOLOGY

The study was used the following equipment: Thermo-hygrometer, Autoclave, Vernier calliper, Centrifuge Desiccator with vacuum pump, Weighing balance, Deep freezer

Environmental monitoring equipment: To measure and record environmental conditions such as temperature and humidity in the experimental room.

Sterilization equipment: Autoclave for sterilizing equipment, bedding materials, and other laboratory supplies to maintain aseptic conditions.

Animal behaviour and physical assessment equipment: Including cages, grids, and tools for observing and evaluating animal behaviour, as well as devices for measuring physical parameters such as body weight and temperature.

Blood and biochemical analysis equipment: Haematology analyse centrifuge, spectrophotometer, and other instruments necessary for performing blood tests and biochemical analyses on collected samples.

Equipment for sample storage: Refrigerators and freezers to store biological samples, ensuring their integrity and preserving their properties for future analysis.

Dose formulation: The required quantity of the test item per dose was weighed out on a watch glass.
The weighed test item was mixed with a portion of the nano-emulgel formulation until it was completely dissolved or dispersed. Both parts, the weighed test item and the nano-emulgel, were thoroughly rinsed to ensure no residue remained.

The mixture was transferred to a final beaker, and the volume was adjusted to the desired dose with the same nano-emulgel formulation.

For the low, mid, and high dose formulations, 100mg, 200mg, and 500mg, respectively, of the test item were added per unit volume of nano-emulgel.
In the case of the reference control formulation, Diclofenac was added at a concentration of 5mg per unit volume of the nano-emulgel formulation, following the study protocol.

The normal control formulation did not contain either the test item or the reference control.
After preparation, the formulations were transferred to labelled beakers and thoroughly mixed.

The beakers containing the formulations were stored appropriately until they were administered to the animal subjects.

It is important to note that all formulations were prepared on the day of use, following the study protocol.

Safety precautions

Personal protective equipment like gloves, gown, head cap, face mask and goggles were used during respective experiment phases.

Signs of toxicity

Throughout the animal experimental study, the health condition of the mice was closely monitored to ensure their welfare. Home cage observations were conducted to document any abnormalities or symptoms exhibited by the mice in their natural habitat. These observations included monitoring for moribund behavior, circling, convulsions, tremors, ataxia (walking backward), hunched posture (kyphosis and lordosis), vocalization, and piloerection. The presence of any of these signs could indicate adverse effects and was taken into consideration for animal welfare considerations.

Fabrication Of Poly Herbal Nanoemulgel

Hydrogel-based nanoemulsion development is the name given to the poly herbal nanoemulgel. They have high epidermal diffusion due to the incorporation of a nanoemulsion drug delivery system within the hydrogel matrix. Numerous researchers have focused on this nanoemulgel in an effort to create a number of medications that can be used to treat various dermis disorders. the topical dosage form for the Nanoemulgel formulation.

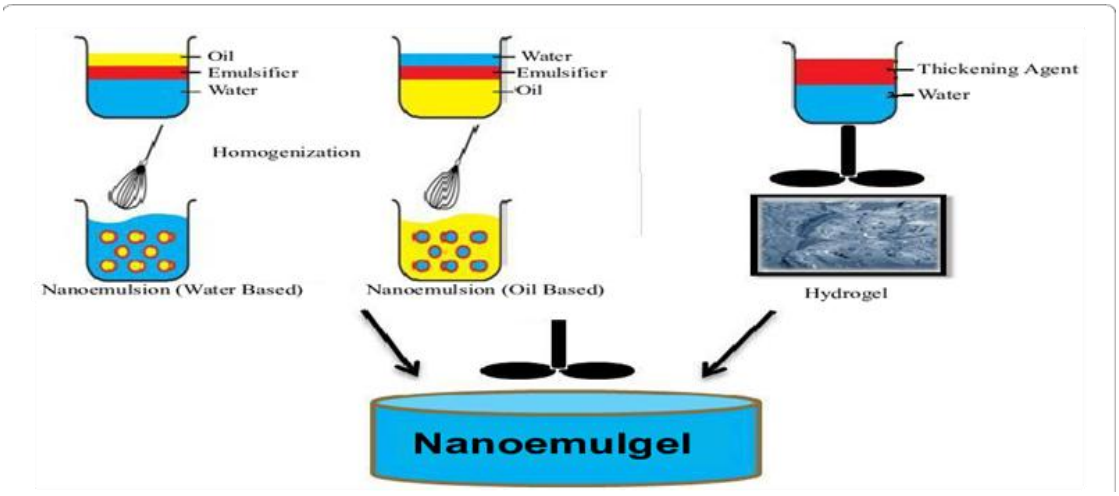


Fig. 2 Process to form Nanoemulgel

Advantage Of Using Polyherbal Nanoemulgel Over Conventional Treatment

The nanoemulgel improves a constant nanoemulsion formulation by reducing the interfacial, surface tension and increasing the viscosity of the aq. phase [18]. Emulsifier and thickener were added to maintain the reliability of the hydrogel. This is for better permeation, viscosity, and stability aimed at the delivery of a dose-loaded topical nanoemulsion. Nanoemulgel drug delivery system, the stability of the nanoemulsion is increased via the supply of oily droplets in the gel system [14]. The nanoemulgel achieves better adhesive characteristics on the dermis layer together with more solubility ability leading a to elevated concentration gradient in the epidermis, which influences the higher diffusion of the medicine into the epidermis as it moves down the gradient. Nanoemulgel also improves patient compliance. Nanoemulgel helps control medicine release by spreading the effects of medicine with shorter half-lives [19]

Importance Of Nanotechnology In Drug Delivery

It can provide better bioavailability. It can provide better stability. In this formulation chances of drug-polymer interactions are less. They are the non-invasive drug delivery carriers that facilitate the transport of drugs to the deeper layer of the skin. They are known for their simplicity in preparation as well as their safety and effectiveness and can be customized to improve the penetration of active drain too the tissue.

Experimental Protocol

On the day of the experiment, mice were randomly selected and grouped based on their stratified body weight, ensuring that the weight of each mouse fell within ±20% of the mean weight within seven days prior to inducing inflammation. Following a seven-day acclimatization period, the mice were then randomly assigned according to the established randomization procedure.

To induce inflammation, a sub-plantar injection of 0.1 mL of a 1% carrageenan suspension in saline was administered to the right hind paw of each mouse, while the left hind paw served as a control. The test item was directly applied to the site without the use of any vehicle. The mice were divided into five groups, with five animals in each group: normal control, reference control (treated with Diclofenac 5mg), low dose (polyherbal formulation 100mg), mid dose (polyherbal formulation 200mg), and high dose (polyherbal formulation 500mg). Paw volume measurements were taken before and after carrageenan injection using a plethysmometer at 0, 1, 2-, 3-, 4-, and 5-hours post-injection. For each group of mice at different time intervals, the percentage increase in paw volume was calculated.

The data obtained from the experiment was examined using one-way ANOVA, followed by Dunnett's test to compare the mean percentage increase in paw volume between the normal control group and the test groups. A significance level of $p < 0.05$ was set to determine statistically significant differences.

Animal Model Selection

The study included a total of 25 animals, which were divided into five different groups. The first group was the Normal Control, which included 5 animals. The second group was the Reference Control, consisting of 5 animals that received Diclofenac at a dose of 5mg. The remaining three groups were experimental groups that received varying doses of a Polyherbal Formulation. The Low Dose group included 5 animals that received 100mg/kg of the Polyherbal Formulation, the Mid Dose group included 5 animals that received 200mg/kg of the Polyherbal Formulation, and the High Dose group included 5 animals that received 500mg/kg of the Polyherbal Formulation. The animals in each group were selected based on their stratified body weight, which fell within ± 20% of the mean body weight. The study aimed to evaluate the efficacy of the Polyherbal Formulation in reducing inflammation, as compared to the Reference Control group and the Normal Control group.

Experimental Conditions

The experiment was conducted in Room number 2, where the environmental conditions were maintained at a temperature range of 19-23°C and a humidity range of 30-70% throughout the study. The temperature and humidity were recorded twice a day to ensure consistency. The animal experimental room was also maintained with 12-15/h air exchanges. The animals were housed with six animals per cage, and the cages were rotated on the rack. The cages were covered with a grid, and autoclaved bedding material was placed inside. The bedding material was autoclaved at 121°C for 20-25 minutes. The animals were provided with laboratory rodent pelleted feed obtained from M/s. VRK Nutritional Solutions, Pune, ad libitum, except on the day of fasting. The study file retained a copy of the recent periodic feed analysis report, and a sample of the diet was retained until the study's completion. The animals were provided with water ad libitum in autoclaved bottles, and the water bottles were changed on a daily basis to ensure the animals had access to clean and fresh water.

These observation techniques were crucial for monitoring the welfare of the mice throughout the experimental study. Any signs of discomfort or illness exhibited by a mouse were reported to the study team, and appropriate measures were taken to alleviate the observed issues. Regular observations also played a vital role in identifying the humane point at which euthanasia of an animal may be necessary to prevent unnecessary pain and distress. Ultimately, these observations played an integral role in ensuring the ethical treatment of the mice used in this experiment.

Table 1: observation table for animal

Home cage observation	**:**	Moribund, circling, convulsion, tremor, ataxia, walking backward, hunched posture, kyphosis, lordosis, vocalisation and piloerection.
Hand held observation	**:**	Self-mutilation, skin-erythema, skin swelling, exophthalmos, lacrimation, chromodacryorrhea, visible mucous membrane for paleness, cyanosis and icterus, dyspnea, nasal discharge, salivation, bleeding from external orifices and diarrhoea.

Table:2- Individual Animal data

			Paw thickness in mm					
Sn.	**Groups**	**Animal No.**	**0h**	**1h**	**2h**	**3h**	**4h**	**5h**
1	Normal Control	1	1.9	1.9	2	1.9	1.9	1.9
		2	1.8	1.8	1.9	1.8	1.8	1.8
		3	1.9	1.9	1.9	2	1.9	1.9
		4	2	1.9	2	2	2	2
		5	1.8	1.8	1.8	1.8	1.8	1.8
2	Reference Control (Diclofenac 5mg)	6	1.9	3.1	3.8	3.9	3.5	2.8
		7	1.8	2.9	3.4	3.8	3.2	2.9
		8	2	3.2	3.5	3.9	3.1	2.8
		9	1.9	3.2	3.9	4.2	3.5	3.1
		10	2.1	3.2	4.1	4.2	3.2	2.4
3	Low Dose (Polyherbal Formulation 100mg)	11	1.8	3.2	4.5	5.1	4.6	3.8
		12	1.7	3.4	4.9	5.2	4.5	3.8
		13	2.1	3.5	4.8	5.3	4.8	3.7

		14	1.8	2.9	5.1	5.4	4.9	3.9
		15	1.9	3.6	4.4	4.9	5.1	3.9
4	Mid Dose (Polyherbal Formulation 200mg)	16	1.8	3.1	4.2	5	4.2	3.5
		17	2	2.9	4.5	4.9	4.1	3.4
		18	1.9	3.4	4.3	4.8	4.4	3.1
		19	1.6	3.5	4.4	5.2	4.3	3
		20	1.9	3.3	4.3	5.1	4.8	2.9
5	High Dose(Polyherbal Formulation 500mg)	21	1.8	3.9	3.9	4.7	3.8	3.1
		22	2.1	3	4.1	4.6	4	2.8
		23	2.3	3.4	4.2	4.5	3.7	3.2
		24	2.1	3.2	3.9	4.1	3.8	2.9
		25	1.7	3.1	3.8	4.3	3.9	3.1

3. RESULTS AND INTERPRETATION

Post-hoc tests (Tukey HSD)

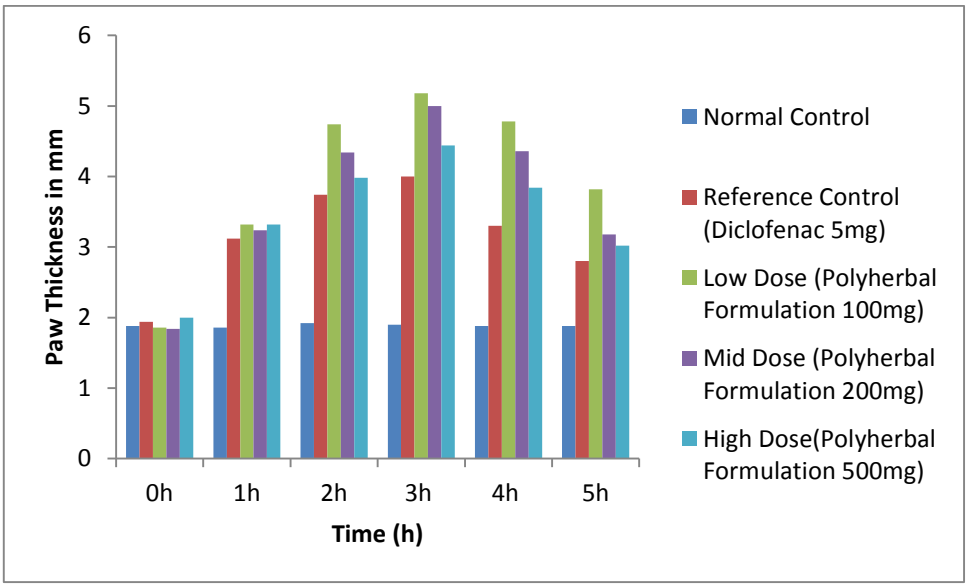
The results of Tukey HSD post-hoc test show that:

The Reference Control group (Diclofenac 5mg) is significantly different from all other groups at all-time points ($p < 0.05$). The Low Dose and Mid Dose groups are significantly different from the Normal Control group at all-time points ($p < 0.05$).

The High Dose group is significantly different from the Normal Control group at the 0h and 5h time points ($p < 0.05$).

Table: 3- The results of ANOVA show a significant effect of treatment on the outcome variable ($F (4, 20) = 17.65$, $p < 0.001$).

Sn.	Groups	0h	1h	2h	3h	4h	5h
1	Normal Control	1.88±0.08	1.86±0.05	1.92±0.08	1.9±0.10	1.88±0.08	1.88±0.08
2	Reference Control (Diclofenac 5mg)	1.94±0.11	3.12±0.13	3.74±0.29	4±0.19	3.3±0.19	2.8±0.25
3	Low Dose (Polyherbal Formulation 100mg)	1.86±0.15	3.32±0.28	4.74±0.29	5.18±0.19	4.78±0.24	3.82±0.08
4	Mid Dose (Polyherbal Formulation 200mg)	1.84±0.15	3.24±0.24	4.34±0.1	5±0.16	4.36±0.27	3.18±0.26
5	High Dose(Polyherbal Formulation 500mg)	2±0.24	3.32±0.36	3.98±0.16	4.44±0.24	3.84±0.11	3.02±0.16



Graph-1: Paw thickness

4. RESULTS

The results of the carrageenan-induced paw edema model showed that the group treated with the high dose of Polyherbal Nanoemulgel exhibited a significant reduction in paw edema compared to the reference control group ($p<0.05$) at 1, 2-, 3-, 4-, and 5-hours post-carrageenan injection. The low and mid-dose groups also showed a reduction in paw edema, but the difference was not statistically significant compared to the reference control group. In the motility test, all groups of mice exhibited normal motility and behavior. There were no significant differences in the number of mice that showed spontaneous movement or reactivity between the groups. This indicates that the administration of the Polyherbal Nanoemulgel did not cause any adverse effects on the mobility or behavior of the mice. In the body weight observation, all groups of mice showed a slight increase in body weight throughout the study period. However, there were no significant differences in the body weight gain between the groups, indicating that the administration of the Polyherbal Nano-emulgel did not affect the growth or development of the mice.

In the home cage observation and hand-held observation, no signs of abnormal behavior or adverse effects were observed in any of the groups of mice. All mice appeared healthy and normal throughout the study period, indicating that the administration of the Polyherbal Nanoemulgel was well-tolerated.

Overall, the results of this study suggest that the Polyherbal Nanoemulgel has anti-inflammatory effects in the carrageenan-induced paw edema model without causing any adverse effects on the mobility, behavior, or growth of the mice. Further studies are needed to investigate the mechanism of action and the long-term safety and efficacy of this formulation.

5. CONCLUSION

Based on these results, we can conclude that the reference control group (Diclofenac 5mg) is the most effective treatment among all groups at all-time points.
The High Dose group is significantly different from the Normal Control group at the 0h and 5h time points, suggesting that it may have some therapeutic effect.
The Low Dose and Mid Dose groups are significantly different from the Normal Control group at all-time points, but not significantly different from each other, suggesting that they may have some therapeutic effect, but not as strong as the Reference Control group.

6. DISCUSSION

Inflammation is a complex biological response of the body's immune system to harmful stimuli such as pathogens, damaged cells, and irritants. Inflammatory response leads to the characteristic signs of redness, swelling, heat, pain, and loss of function at the affected site. Inflammation plays a crucial role in the pathogenesis of several acute and chronic diseases such as arthritis, asthma, and cardiovascular diseases. Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used to manage inflammation and associated pain. However, long-term use of NSAIDs is associated with several adverse effects such as gastrointestinal bleeding, renal toxicity, and cardiovascular complications.

To overcome the limitations of conventional NSAIDs, researchers are exploring natural and herbal sources as potential anti-inflammatory agents. Nyctanthes arbor-tristis, commonly known as the night-flowering jasmine, has been traditionally used in Ayurvedic medicine to treat inflammatory diseases. Aloe vera is another plant widely used for its anti-inflammatory properties. A polyherbal formulation containing Nyctanthes arbor-tristis and Aloe vera was developed as a nanoemulgel for topical administration to treat inflammation.

The carrageenan model paw edema model in male Swiss albino mice is a widely used animal model to study the acute inflammation response. In this study, the polyherbal nanoemulgel was evaluated for its anti-inflammatory activity using the carrageenan model paw edema model. The results showed that the polyherbal nanoemulgel significantly reduced the paw edema volume in a dose-dependent manner as compared to the normal control group. The high dose of the nanoemulgel (500mg) showed the highest anti-inflammatory activity.

The anti-inflammatory activity of the polyherbal nanoemulgel could be attributed to the presence of several bioactive compounds in Nyctanthes arbor-tristis and Aloe vera. Nyctanthes arbor-tristis contains several flavonoids such as apigenin, luteolin, and quercetin, which have been reported to possess anti-inflammatory activity. Aloe vera contains several compounds such as aloin, aloe-emodin, and chrysophanol, which have been reported to have anti-inflammatory activity.
The nanoemulgel formulation offers several advantages over conventional dosage forms such as creams and gels. The small droplet size of the nanoemulsion enhances the surface area of the active ingredients, leading to better skin penetration and increased bioavailability. The use of a nanoemulsion also offers improved stability,

prolonged shelf life, and reduced skin irritation as compared to conventional dosage forms.

In conclusion, Nyctanthes arbor-tristis and Aloe vera were used in a polyherbal nano-emulgel that demonstrated notable anti-inflammatory effect in a carrageenan model paw edoema model in male Swiss albino mice.
The nanoemulgel formulation is superior to traditional dose forms in a number of ways, and it may one day replace traditional NSAIDs as a treatment for pain and inflammation. The effectiveness and safety of the nanoemulgel in chronic inflammatory models and on humans must be further investigated.

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7. REFERENCES

1. Reddi, D., Curran, N., & Stephens, R. An introduction to pain pathways and mechanisms. British Journal of Hospital Medicine,2013; 74(Sup12), C188–C191
2. International Association for the Study of Pain. IASP Terminology. 2020
3. Moayedi M, Davis KD. Theories of pain: From specificity to gate control. J Neurophysiol 2013; 109:5-12.
4. Świeboda P, Filip R, Prystupa A, Drozd M. Assessment of pain: types, mechanism and treatment. Pain. 2013;2(7).
5. Astuti K. W, Wijayanti N. P. A. D, Yustiantara P. S, Laksana K. P, Putra P. S. A. Anti-Inflammatory Activity of Mangosteen (Garcinia Mangostana Linn.) Rind Extract Nanoemulgel and Gel Dosage Forms. Biomed Pharmacol J 2019;12(4).
6. Abdallah M. H., Abu Lila, A. S., Unissa, R., Elsewedy, H. S., Elghamry, H. A., & Soliman, M. S. (2021). Preparation, characterization and evaluation of anti-inflammatory and anti-nociceptive effects of brucine-loaded nanoemulgel. Colloids and Surfaces B: Biointerfaces, 205
7. P. van Hoogevest, X. Liu, A. Fahr, Drug delivery strategies for poorly water-soluble drugs: the industrial perspective, Expert Opin. Drug Deliv. 8 (2011) 1481–1500.
8. M.C. Pena-Juarez, O.R. Guadarrama-Escobar, J.J. Escobar-Chavez, Transdermal delivery systems for biomolecules, J. Pharm. Innov. (2021).
9. M. Isaac, C. Holvey, Transdermal patches: the emerging mode of drug delivery system in psychiatry, Ther. Adv. Psychopharmacol. 2 (2012) 255–263.
10. K.S. Paudel, M. Milewski, C.L. Swadley, N.K. Brogden, P. Ghosh, A.L. Stinchcomb, Challenges and opportunities in dermal/transdermal delivery, Ther. Deliv. 1 (2010) 109–131.
11. Eid AM, El-Enshasy HA, Aziz R, Elmarzugi NA (2014) Preparation, Characterization and Anti-Infammatory Activity of Swietenia macrophylla Nanoemulgel. Journal of Nanomedicine Nanotechnology, Vol. 5: Pgno.190
12. Chen H, Khemtong C, Yang X, Chang X, Gao J. Nanonization strategies for poorly water-soluble drugs. Drug Discov Today. 2011 Apr;16(7-8):354-60.
13. Shakeel F, Ramadan W, Ahmed MA. Investigation of true nanoemulsions for transdermal potential of indomethacin: characterization, rheological characteristics, and ex vivo skin permeation studies. J Drug Target. 2009 Jul;17(6):435-41.
14. Bhadra, P. (2020). Efficacy of some compounds isolated from Nyctanthes arbortristis Linn. on human and plant diseases as revealed from in silico analysis. Indian Journal of Natural Sciences, 10(60), 20833-20839.
15. Mishra, A. K., Tiwari, K. N., Saini, R., Kumar, P., Mishra, S. K., Yadav, V. B., & Nath, G. (2020). Green synthesis of silver nanoparticles from leaf extract of Nyctanthes arbor-tristis L. and assessment of its antioxidant, antimicrobial response. Journal of Inorganic and Organometallic Polymers and Materials, 30(6), 2266-2278.
16. Jamdagni, P., Khatri, P., & Rana, J. S. (2018). Green synthesis of zinc oxide nanoparticles using flower extract of Nyctanthes arbor-tristis and their antifungal activity. Journal of King Saud University-Science, 30(2), 168-175.
17. Gond, S. K., Mishra, A., Verma, S. K., Sharma, V. K., & Kharwar, R. N. (2020). Synthesis and characterization of antimicrobial silver nanoparticles by an endophytic fungus isolated from Nyctanthes arbor-tristis. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 90(3), 641-645.
18. Karan, B. N., Maity, T. K., Pal, B. C., Singha, T., & Jana, S. (2019). Betulinic Acid, the first lupane-type triterpenoid isolated via bioactivity-guided fractionation, and identified by spectroscopic analysis from leaves of Nyctanthes arbor-tristis: its

potential biological activities in vitro assays. Natural product research, 33(22), 3287-3292.

19. Sharma, L., Dhiman, M., Singh, A., & Sharma, M. M. (2021). Biological synthesis of silver nanoparticles using Nyctanthes arbor-tristis L.: A green approach to evaluate antimicrobial activities. Materials Today: Proceedings, 43, 2915-2920.
20. Gupta, A., Koirala, A. R., Gupta, B., & Parajuli, N. (2019). Improved method for separation of silver nanoparticles synthesized using the Nyctanthes arbor-tristis shrub. Acta Chemica Malaysia, 3(1), 35-42.
21. Rath, S. C., Seth, S., Mishra, S. K., Yadav, P. K., Gupta, A. K., & Panigrahi, J. (2020). Genetic homogeneity assessment of in vitro-regenerated plantlets of Nyctanthes arbor-tristis L. and comparative evaluation of bioactive metabolites and antioxidant activity. In Vitro Cellular & Developmental Biology-Plant, 56(1), 72-87.
22. Mishra, A. K., Tiwari, K. N., Mishra, P., Tiwari, S. K., Mishra, S. K., & Saini, R. (2019). Effect of cytokinin and MS medium composition on efficient shoot proliferation of Nyctanthes arbor-tristis L. through cotyledonary node explant and evaluation of genetic fidelity and antioxidant capacity of regenerants. South African Journal of Botany, 127, 284-292.
23. Balasubramanian, M., & Murali, K. R. (2020). Biosynthesis of zinc ferrite (ZnFe2O4) nanoparticles using flower extract of nyctanthes arbor-tristis and their photocatalytic activity. Ferroelectrics, 555(1), 1-14.
24. Rawat H., Verma, Y., Ayesha, N. S., Negi, N., Pant, H. C., Mishra, A., ... & Gaurav, N. (2021). Nyctanthes arbor-tristis: a traditional herbal plant with miraculous potential in medicine. International Journal of botany Studies, 6, 427-440.