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Cytotoxic Evaluation of Nanoemulsion Prepared from Aqueous Extract of *Acorus Calamus.*

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KEYWORDS

Cytotoxic property; Acorus calamus; Nanoemulsion; Pain pathway

ABSTRACT:

The study aims to formulate Acorus calamus nanoemulsion and check for cytotoxic properties. To prepare the nanoemulsion, 10 mg of Acorus calamus powder was dissolved in 10 mL of water. Subsequently, 60 mL of palm oil and 30 mL of Tween 80 were added to the mixture to form an oil-in-water emulsion. The resulting mixture was subjected to ultrasonication for 60 min using an ultrasonic processor. The formation of a milky white solution indicated the successful creation of the nano-emulsion. During the optimisation of the nano-emulsion, it was observed that as the volume of the Acorus calamus nano-emulsion increased, the particle size distribution also increased, ranging from 140 to 160 nm when the volume reached 100%. Beyond this volume, the particle size gradually decreased, stabilising at approximately 240 nm. The cytotoxicity evaluation revealed that the Acorus calamus nano-emulsion exhibited minimal cytotoxic activity, indicating its safety for topical applications. Notably, the nano-emulsion demonstrated enhanced penetration through human epithelial cells when the particle size was less than 100 microns, suggesting its potential to manage trigeminal neuralgia when applied topically.

1. Introduction

The trigeminal nerve is the 5th cranial nerve in the face region, which is divided into 3 branches: Ophthalmic (V1), maxillary (V2), and mandibular (V3) divisions. The maxillary branch also comprises the largest branch of the trigeminal nerve. The International Association of Pain defines trigeminal neuralgia as a sudden, usually unilateral, severe, brief stabbing, recurrent pain episode in the distribution of one or more branches of the trigeminal nerve (1). The pain is of more severe onset and gradual in duration, triggered while washing the face, speaking, touching, brushing, and even by a puff of wind. It is also known as Fothergill disease or suicidal disease, which involves the patient's quality of life as well as their day-to-day activities (2). There is a deterioration in the quality of life. The pain lasts for 10 seconds to 1 minute only, and the pain doesn't cross the midline of the face (Mueller et al. 2011). In adults, the mean age affected ranges from 53 to 58 years of age, with a slightly female predilection over males, with a ratio of 3:2. Trigeminal neuralgia is of 3 types: primary or classic, secondary, and idiopathic. Primary or classical neuralgia is characterized by neurovascular conflict, where a blood vessel contacts the trigeminal nerve. Secondary trigeminal neuralgia is due to cysts or tumours that involve the trigeminal nerve, like schwannoma, multiple sclerosis, etc. Idiopathic trigeminal neuralgia is characterized by an apparent cause. The main aim of the study is to decrease the pain intensity and increase the quality of life of affected patients (3).

Carbamazepine is both an anticonvulsant and an antiepileptic drug, considered a gold standard in the treatment of trigeminal neuralgia. Along with carbamazepine, ox-carbamazepine and baclofen are also given in combination. Carbamazepine has a calcium channel inhibitory action, which is involved in the conduction of the pain pathway, thereby inhibiting pain activity. It can be given at a maximum dosage of 1200

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mg per day. Although it has the property of calcium channel inhibition, it has a common side effect of bone marrow suppression, causing agranulocytosis and drowsiness. Agranulocytosis is more dangerous when the total neutrophil count decreases to 100 cells/mm^3. Dosage ranges from 100 mg to 1200 mg per day. Due to the most common side effects of carbamazepine, such as agranulocytosis, there is a need for an alternative to minimize the side effects of carbamazepine.

Maragathavalli et al. conducted a study on the incidence of trigeminal neuralgia and found that 83.3%, whereas the incidence of other orofacial neuralgia was found to be 16.7%. The majority of the study population was in the age group of 41-70 years. Males (54.1%) dominated the study population over females (45.8%) (4). Maheswari et al. conducted a study on the prevalence of trigeminal neuralgia in a private institution and found that the majority of the affected individuals are in the age groups of 30-60 years, and there is a slightly greater female predilection than males. These people are mostly prescribed carbamazepine along with mecobalamin in the management of trigeminal neuralgia (5). Arvind et al. conducted a study on nerve involvement and distribution of pain in trigeminal neuralgia and found that the most common site of involvement is the right side rather than the left side, and it is most common in the male population (6).

Acorus calamus is a well-known medicinal plant used in traditional medicine for the management of liver and hepatobiliary carcinomas. It has a scientific name of vacha. A wide variety of metabolites have been used previously in the management of cancers. Root rhizomes of the *Acorus calamus* have anticancer, antioxidant, and neuroprotective properties by inhibiting the action of calcium channels, which are mainly responsible for the pain conduction pathway (7). By inhibiting the calcium channel inhibitory action, it greatly reduces the pain intensity as well. Before moving on to the *Acorus calamus*, there is a need to check for cytotoxic properties and antioxidant properties. The main aim of the study is to prepare the nanoemulsion of the *Acorus calamus* and assess the cytotoxic activity of the prepared nanoemulsion.

2. Materials and methods

2.1. Materials

Tween 80 was purchased from Loba Chemie Private Ltd., Mumbai, India, and palm oil was purchased from a natural-based plant company in Chennai, India. *Acorus calamus* rhizome was collected from a naturally available herbal centre located in Madurai, India.

2.2. Preparation of nano-emulsion

The Soxhlet device was used to carry out the extraction procedure. Here, bioactive components were extracted aqueously from a dried sample (10 g). Rotary evaporation was used to evaporate the extract and collect the powder. The nano-emulsions were prepared by dispersing 10 mg of powder into water and subsequently 60 mL of palm oil and 30 mL of Tween 80, to create an oil-in-water emulsion. The reaction mixture was sonicated for 60 min, and successful milky white formation indicates nano-emulsion formation.

2.3. Cytotoxicity Assay

Cytotoxic activity of the *Acorus calamus* was evaluated using the 3-[4,5-dimethylthiazol-2-yl 2,5-diphenyl tetrazolium bromide (MTT) test to determine its effect on the proliferation of human epithelial cells (10). The cells with a passage number of 8 were subsequently grown in a humidified chamber at 37 °C and 5% CO_2. The resulting cells were then transferred at a concentration of 5 x 10^4 cells/well onto 96-well culture plates. *Acorus calamus* was added to the cells at different doses (30 mg/L). Following a 24-hour incubation phase with *Acorus calamus*, each well received 100 µL of MTT solution after the media was drained. To promote the creation of formazan crystals, the cells were then cultured for a further four hours. After that, the formazan crystals were dissolved in 100 µL of dimethyl sulfoxide (DMSO), and the intensity of the resultant mixture was quantified using a microplate reader at a wavelength of 570 nm.

3. Results

3.1. Characterisation of the nanoemulsion

The particle size distribution (Fig. 1a) is used to depict the particle size at which the nanoemulsion is dimensionally stable. The particle size distribution analysis demonstrated a gradual increase in volume

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percentage with increasing particle size, reaching a maximum at 160 nm. The volume percentage increased from 15% at 20 nm to a peak value of 100% at 160 nm, indicating that the majority of particles were concentrated around this size.

Beyond 160 nm, a progressive decline in volume percentage was observed, decreasing to 10% at 240 nm. The distribution pattern was unimodal, with a predominant particle size range between 100 nm and 180 nm.

These findings indicate that the formulated system exhibits a nanoscale distribution with the majority of particles centered around 140–160 nm, suggesting a relatively uniform particle size distribution suitable for enhanced stability and effective topical delivery.

The zeta potential (Fig. 1 b) is used to determine whether the nano-emulsion prepared is dimensionally stable or not. The bulk of particles have near-neutral surface charge, according to the zeta potential study, which showed a strong and narrow peak centered around 0 mV. A consistent surface charge across the particle population was suggested by the distribution curve's extreme symmetry and restricted range.

The peak's high intensity indicates a uniform and uniform particle dispersion. However, low electrostatic stabilization is indicated by the zeta potential value being near neutral, which may eventually make the formulation more susceptible to particle aggregation.

The formulation shows a monodisperse zeta potential distribution overall, but the nearly zero zeta potential indicates that steric stabilizing mechanisms (such as polymers or surfactants) may be more important for stability than electrostatic repulsion. The zeta potential value is raised when the particle size is at 31nm; the normal value of less than -30 up to +30 the value is unstable. However, the nanoemulsion of the *Acorus calamus* is 31, which shows that the nanoemulsion is dimensionally stable.

SEM image (Fig. 1c) shows the arrangement of the particles in the nanoemulsion. The SEM micrograph (×10,000 magnification; scale bar: 1 µm) showed that the particles had reasonably smooth surfaces and a mostly spherical to ¹–spherical shape. According to the results of the particle size distribution, the particles seemed to be well-formed and nanosized.

However, there was some particle aggregation and clustering, which might be explained by the low zeta potential levels and interparticle interactions. Instead of a totally discrete dispersion, the particles were dispersed unevenly across the surface, generating localized clusters.

The previous particle size analysis was supported by the individual particle sizes, which seemed to be within the nanometer range (about <200 nm). Additionally, the surface topology revealed a compact and dense structure, indicating successful nano-system formulation and stabilization.

3.2. Cytotoxicity Assay

The cell viability assay demonstrated that all test samples maintained high cell viability values close to 100%, indicating minimal cytotoxicity. The control group exhibited approximately 100% viability, serving as the baseline for comparison.

Among the test groups, Extract 1 mL showed slightly reduced viability (~98–99%), while Extracts 2 mL, 3 mL, and 4 mL maintained values around 97–99%. Extract 5 mL showed a marginal decrease (~96–97%), though still within a highly acceptable range.

Error bars across all groups were minimal, indicating low variability and good reproducibility of the results.

Overall, the findings suggest that the formulation is biocompatible and non-toxic, as all concentrations tested maintained >95% cell viability, with no significant reduction compared to the control.

4. Discussion

The results of this study showed that the nanoemulsion of *Acorus calamus* has a minimal amount of cytotoxic activity through the particle size distribution. The dynamic light scattering (DLS) method is a technique that can be used to determine the size distribution profile of small particles in suspension or polymers in solution. In the scope of DLS, temporal fluctuations are usually analyzed using the intensity or photon autocorrelation function (also known as photon correlation spectroscopy – PCS or quasi-elastic light scattering – QELS). Rajkumar et al. tested the cytotoxicity in the *Acorus calamus* Rhizome and observed that cytotoxicity is seen in the *Acorus calamus*, hence it is involved in the treatment of cancers.

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In the current study, it is shown that *Acorus calamus* nanoemulsion was prepared using the Soxhlet apparatus. Here, a 10 g dried sample was used for the aqueous extraction of bioactive compounds. The extract was evaporated, and the powder was collected by using rotary evaporation (8).

The findings of this investigation demonstrate that the SEM image of the *Acorus calamus* nanoemulsion was spherical. In my study, the focus is on reducing the pain conduction elicited by trigeminal neuralgia. Rhizomes of the *Acorus calamus* have neuroprotective properties as well as antioxidant and calcium channel inhibitory action.

Another study investigated the ameliorative effect of ethanolic extract from the *Acorus calamus* in the CCI model. Pretreatment of the *Acorus calamus* attenuated CCI-induced development of histopathological, biochemical, and behavioural alterations dose-dependently, which is comparable to that of the pregabalin pretreated group (9).

Kumar et al. (10) tested the *Acorus calamus* rhizome in rats for antimicrobial properties, and they found that Acorus calamus has a better anti-bacterial effect when it is used systemically in rats, and they observed that bacterial cells were excreted in the faeces of rats.

This may be due to its potential anti-oxidative, neuroprotective, and calcium channel modulatory effects on the *Acorus calamus* (11). My current study suggests that *Acorus calamus-based* nano-emulsion has minimal cytotoxic activity when combined with palm oil extract. When compared to the trolox-positive group, the nano-emulsion of *Acorus calamus* has higher penetration when the size is less than 100 microns into the human epithelial cells.

In my study, the focus is on reducing the pain conduction elicited by trigeminal neuralgia. Rhizome of the *Acorus calamus* has neuroprotective properties as well as antioxidant and calcium channel inhibitory action. *Acorus calamus* is used as a lotion as well as an anticancer drug in the management of hepatobiliary carcinoma in Siddha medicine. Pretreatment with *Acorus calamus* significantly increased the behavioral (i.e. hyperalgesia and allodynic pain sensation) changes and decreased thiol barbituric acid reactive substances (TBARS), total calcium levels besides increased the glutathione (GSH) levels in the sciatic nerve tissue when compared with the normal control group on vincristine-induced neuropathic pain model in rats, that may be due to its potential of neuroprotective, antioxidant and calcium channel inactivation.

The nanoemulsion has a head, body part, and tail. The head part comprises a hydrophobic head part, and the tail is a hydrophilic part. There, the nanoemulsion was trapped inside the particle. The limitations of the study are assessment of the same sample in different titers is difficult, and different volumes of extracts might show some processing errors during the study, which might alter the effects to a certain extent. There is a need for more studies to prove the effects of nanoemulsion on the *Acorus calamus*.

5. Conclusion

Acorus calamus nanoemulsion has a minimal amount of cytotoxic activity, and it is quite safe when it is applied topically. Nanoemulsion of the *Acorus calamus* has higher penetration when the size is less than 100 microns into the human epithelial cells. So it is more useful in the management of trigeminal neuralgia when it is applied topically. This novel formulation of the *Acorus calamus* can safely be used as an adjunct therapeutic agent in the management of Trigeminal neuralgia. Nanoemulsion particle sizes up to 100 microns will penetrate into the human epithelial cells when it is applied topically. As there are fewer studies and pieces of evidence, there is a need for more studies to evaluate the pharmacological actions and Antioxidant properties of nanoemulsion *Acorus calamus.*

6. REFERENCES

1. Zakrzewska JM, Wu J, Mon-Williams M, Phillips N, Pavitt SH. Evaluating the impact of trigeminal neuralgia. Pain [Internet]. 2017 Jun;158(6):1166–74. Available from: http://dx.doi.org/10.1097/j.pain.0000000000000853

2. Sjaastad O, Bakketeig LS. The rare, unilateral headaches. Vågå study of headache epidemiology. J Headache Pain [Internet]. 2007 Feb;8(1):19–27. Available from: https://doi.org/10.1007/s10194-006-0292-4

3. Koopman JSHA, Dieleman JP, Huygen FJ, de Mos

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M, Martin CGM, Sturkenboom MCJM. Incidence of facial pain in the general population. Pain [Internet]. 2009 Dec 15;147(1-3):122–7. Available from: https://doi.org/10.1016/j.pain.2009.08.023

4. Incidence Trigeminal Neuralgia University Hospital Setting -A Retrospective Analysis.

5. Murugesan I, T N U, Ramalingam K. A retrospective analysis of medical management strategies for trigeminal neuralgia: An institutional review. Cureus [Internet]. 2024 Sep;16(9):e69258. Available from: http://dx.doi.org/10.7759/cureus.69258

6. Priyanka, Arvind M, Priyanka. A retrospective study on side of nerve involvement and distribution of pain in patients with trigeminal neuralgia. Indian J Public Health Res Dev [Internet]. 2020 Jan 1;11(1):410. Available from: https://doi.org/10.37506/v11%2Fi1%2F2020%2Fijphrd%2F194147

7. Rajaulla Naikwadi S. An overview on Acorus Calamus (sweet flag): A gift from nature. Int J Sci Res (Raipur) [Internet]. 2024 Aug 5;13(8):1279–82. Available from: http://dx.doi.org/10.21275/sr24820103501

8. Weislow OS, Kiser R, Fine DL, Bader J, Shoemaker RH, Boyd MR. New soluble-formazan assay for HIV-1 cytopathic effects: application to high-flux screening of synthetic and natural products for AIDS-antiviral activity. J Natl Cancer Inst [Internet]. 1989 Apr 19;81(8):577–86. Available from: https://doi.org/10.1093/jnci/81.8.577

9. Hansen MB, Nielsen SE, Berg K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J Immunol Methods [Internet]. 1989 May 12;119(2):203–10. Available from: https://doi.org/10.1016/0022-1759(89)90397-9

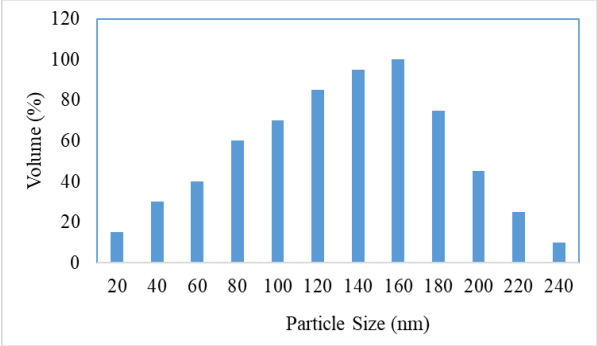
10. Nandakumar S, Menon S, Shailajan S. A rapid HPLC-ESI-MS/MS method for determination of β-asarone, a potential anti-epileptic agent, in plasma after oral administration of Acorus calamus extract to rats. Biomed Chromatogr [Internet]. 2013 Mar;27(3):318–26. Available from: http://dx.doi.org/10.1002/bmc.2794

11. Rajkumar, Gunjan G, Kumar RA, Lazăr M. Evaluation of Cytotoxic Potential of Acorus calamus Rhizome. Ethnobotanical Leaflets [Internet]. 2009;2009(7):2. Available from: https://r.search.yahoo.com/_ylt=Awrx.wsw3QFoCQIAeEK7HAx.;_ylu=Y29sbwNzZzMEcG9zAzIEdnRpZAMEc2VjA3Ny/RV=2/RE=1746162225/RO=10/RU=https%3a%2f%2fopensiuc.lib.siu.edu%2febl%2fvol2009%2fiss7%2f2%2f/RK=2/RS=c_kgvSJZ.bdT6TYGhIMFSSYQFLE-

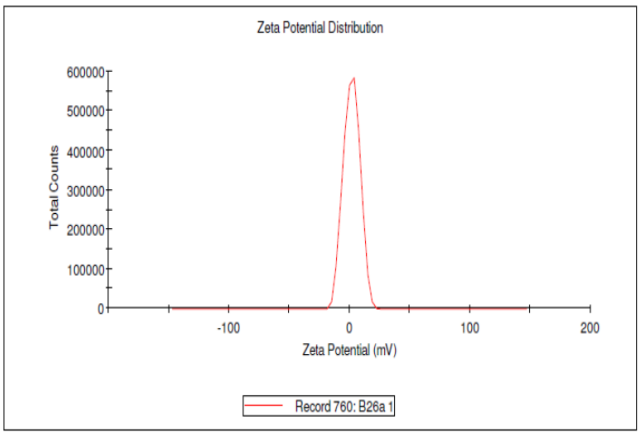
CYTOTOXICITY OF ACORUS CALAMUS NANOEMULSION

Fig. 1. a) Particle size distribution, b) Zeta potential, and c) SEM image of prepared emulsion.

1a) Particle size distribution



1b) Zeta potential



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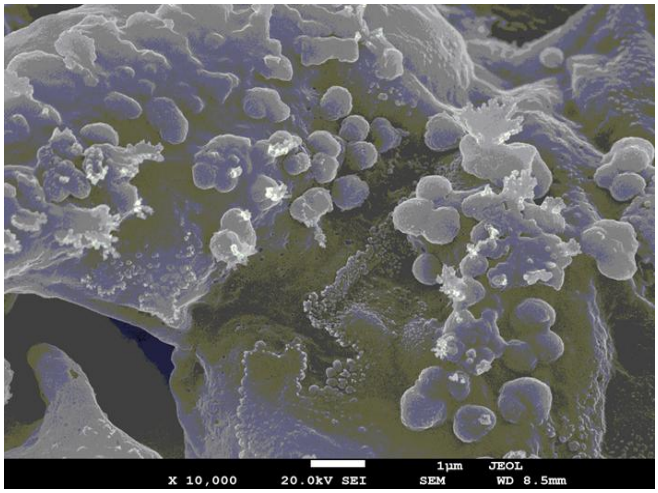
1c) SEM image



2) Cytotoxicity assay