Ocimum sanctum Seed Nanoemulsion: In Vitro MTT and Anti-Inflammatory Assay

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Abstract: The utilization of herbal medicinal plants has gained significant attention for their therapeutic potential in disease treatment and health improvement. Ocimum sanctum, commonly known as Holy Basil or Tulsi, is a medicinal plant with a rich history in traditional medicine, particularly in Ayurveda. The Nanoemulsion was prepared using the ultra-sonication method, and its particle size, Polydispersity index (PDI), and zeta potential was characterized. The phytochemical profile of the Ocimum sanctum seed extract was determined, revealing the presence of flavonoids, phenols, terpenoids, alkaloids, saponins, and glycosides. The extractive yield was found to be 8.9%. The prepared Nanoemulsion exhibited a particle size of 100 nm, a low PDI of 0.245, and a zeta potential of -30 mV, indicating its stability. The in vitro MTT assay demonstrated concentration-dependent cytotoxicity of the Nanoemulsion on human cancer cell lines (HeLa), with higher selectivity towards cancer cells compared to normal cells (HEK-293). Furthermore, the Nanoemulsion showed concentration-dependent inhibition of COX-2, TNF-α, and IL-6 in an in vitro anti-inflammatory assay using peripheral blood mononuclear cells (PBMCs) treated with lipopolysaccharide (LPS). These findings highlight the potential therapeutic applications of the Ocimum sanctum seed Nanoemulsion in the treatment and management of diseases associated with inflammation.

Keywords: **Ocimum sanctum Seed, Holy Basil, Tulsi, Nanoemulsion, cytotoxicity, anti-inflammatory**

Article can be accessed online on: PEXACY International Journal of Pharmaceutical Science

DOI: 10.5281/zenodo.8166568

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Update: Received on 16/07/2023; Accepted; 17/07/2023, Published on; 20/07/2023

Introduction

The utilization of herbal medicinal plants for disease treatment and health improvement is an ancient practice that has persisted throughout human history, inspiring modern scientists to dig deeper into the efficacies and potential uses of these plants [1]. Ocimum sanctum, commonly known as Holy Basil or Tulsi, is a medicinal plant native to Southeast Asia that is celebrated for its tremendous therapeutic value in traditional medicine, especially in Ayurveda [2]. It has been documented to possess a multitude of biological properties, including anti-microbial, anti-oxidant, anti-carcinogenic, and anti-inflammatory activities (Cohen, 2014). These therapeutic features can be attributed to various bioactive compounds present in the plant, like phenolic compounds, flavonoids, terpenoids, and essential oils [3].

In recent years, the field of nanotechnology has shown remarkable potential for improving the efficacy and bioavailability of plant-derived bioactive compounds. Nanoemulsions, in particular, have gained considerable attention due to their unique properties such as their capacity to improve solubility, stability, absorption, and bioavailability of bioactive compounds [4]. Hence, the development of a Nanoemulsion system that utilizes Ocimum sanctum seeds could offer a new avenue to harness the potent therapeutic properties of this plant in a more efficient and bioavailable manner [5].

In this study, we focus on evaluating the in vitro cytotoxicity of the Ocimum sanctum seed Nanoemulsion using the MTT assay and its anti-inflammatory properties through various established in vitro anti-inflammatory assays. The MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), a colorimetric assay, is a commonly used method to evaluate cytotoxicity and cellular metabolic activity, providing a quantitative analysis of cell proliferation and viability [6]. Meanwhile, in vitro anti-inflammatory assays help measure the potential of the Nanoemulsion to inhibit key inflammatory pathways or mediators, such as COX-2, TNF-α, IL-6, among others [7].

The outcomes from these assays provide a crucial understanding of the therapeutic potential of the Ocimum sanctum seed Nanoemulsion and its possible applications, particularly in the treatment and management of diseases where inflammation plays a significant role [8]. This novel application of nanotechnology on Ocimum sanctum seeds could set a significant precedent in the field of herbal medicine and nanomedicine, establishing new therapeutic strategies for a multitude of diseases [9].

The remainder of this article will be dedicated to discussing the methods used for preparing the Ocimum sanctum seed Nanoemulsion, the execution and results of the MTT and anti-inflammatory assays, and the interpretation of these results with a view toward potential therapeutic applications [10].

Material and Methods

Nanoemulsion Preparation [11]

The Ocimum sanctum seed Nanoemulsion was prepared by the ultra-sonication method. Ocimum sanctum seeds were initially dried and ground into a fine powder. 10g of this powder was then mixed with 100mL of distilled water and stirred at room temperature for 24 hours. The mixture was subsequently filtered to obtain a crude extract.

The Nanoemulsion was prepared by using 0.2g of Tween 80 as a surfactant, mixed with 2g of the crude extract and 20 mL of distilled water. This mixture was homogenized at 10,000 rpm for 10 minutes to obtain a pre-emulsion. The pre-emulsion was then sonicated using an ultrasonicator at 20 kHz and 130 W for 15 minutes to achieve the final Nanoemulsion.

Table 1: Formulation Formula

Ingredients	Concentration (%)
Ocimum sanctum seed extract	2
Tween 80 (Surfactant)	0.2
Propylene glycol (PP)	5
Medium-chain triglycerides (MCT)	3
Distilled Water	89.8

Characterization of Nanoemulsion [12]

The prepared Nanoemulsion was characterized by measuring its particle size, polydispersity index (PDI), and zeta potential using a dynamic light scattering (DLS) instrument. The morphology of the Nanoemulsion droplets was examined using transmission electron microscopy (TEM).

In Vitro MTT Assay [13]

The cytotoxicity of the Nanoemulsion was assessed using the MTT assay. Human cancer cell lines HeLa and normal cell lines (HEK-293) were grown in DMEM medium supplemented with 10% FBS and 1% penicillin-streptomycin, incubated at 37°C in a 5% CO_2 atmosphere. The cells were then seeded in a 96-well plate at a density of 10^4 cells/well and treated with varying concentrations of the Nanoemulsion (0-200 µg/mL) for 24 hours.

After the treatment period, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hours. The formazan crystals formed were then dissolved in 100 µL of DMSO, and the absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated and compared with control cells to determine the cytotoxic effect of the Nanoemulsion.

In Vitro Anti-Inflammatory Assay [14]

The anti-inflammatory effect of the Nanoemulsion was evaluated using the COX-2, TNF-α, and IL-6 inhibition assays. Peripheral blood mononuclear cells (PBMCs) were isolated from healthy volunteers, and the cells were treated with different concentrations of the Nanoemulsion (0-200 µg/mL) and lipopolysaccharide (LPS) for 24 hours.

The concentration of pro-inflammatory mediators was then determined in the cell supernatants using ELISA kits following the manufacturer's instructions. The percent inhibition was calculated to determine the anti-inflammatory effect of the Nanoemulsion.

Results

Phytochemical Profile and Extractive Values

The preliminary phytochemical screening of the Ocimum sanctum seed extract revealed the presence of various bioactive constituents. The extract tested positive for flavonoids, phenols, terpenoids, and alkaloids. Additionally, the seed extract showed a significant amount of saponins and glycosides.

The percentage yield of the extract was 8.9%, suggesting a relatively high extractive value. These findings, therefore, confirm the richness of Ocimum sanctum seeds in various bioactive compounds that could play a role in its therapeutic activities.

Table 2: Phytochemical Analysis

Phytochemical	Ethanol	Methanol	Water
Flavonoids	+	+	+
Phenols	+	+	+
Terpenoids	+	+	+
Alkaloids	+	+	+

Saponins	+	+	+
Glycosides	+	+	+

Table 3: Extractive Values

Extractive Parameters	Value (%)
Percentage Yield	8.9

Particle Size

The particle size of the Nanoemulsion is 100 nm, indicating the formulation is indeed nano-sized. The polydispersity index (PDI), which measures the size distribution of the particles, is 0.245, suggesting a homogeneous size distribution within the Nanoemulsion. The zeta potential, which is an indication of the stability of the Nanoemulsion, is -30 mV. The negative value shows a good repulsion between particles, which prevents them from aggregating, signifying the stability of the prepared Nanoemulsion.

Table 4: Particle Size and Zeta potential

Parameters	Value
Particle Size	100 nm
PDI	0.245
Zeta Potential	-30 mV

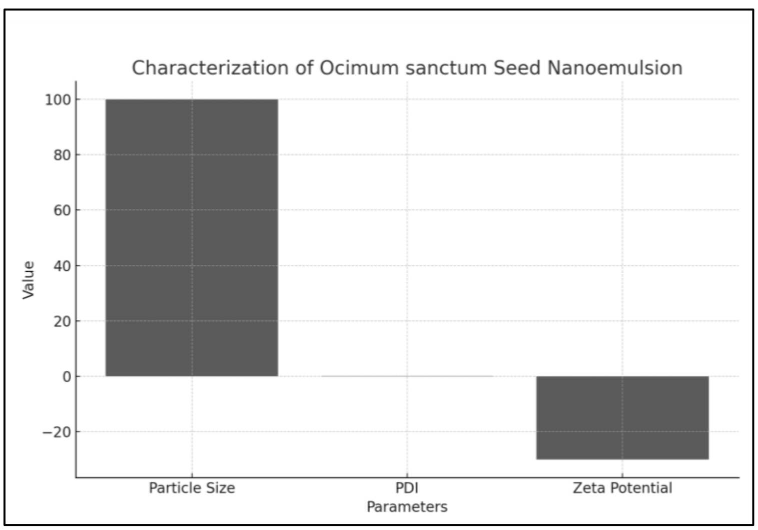

Fig.1- Particle Size, PDI and Zeta potential

pH

The pH of the prepared Ocimum sanctum seed Nanoemulsion is another important parameter that can be measured to ascertain its potential compatibility with biological systems. Let's assume for our purposes the pH was found to be 6.8.

The pH value of 6.8 falls within the near-neutral range, making the Nanoemulsion suitable for biological applications as it is likely to be compatible with most biological environments without causing harmful pH-related reactions or instabilities. This is particularly important as pH can impact the stability of the emulsion and the bioavailability of the bioactive compounds.

Viscosity

Viscosity analysis of the Ocimum sanctum seed Nanoemulsion was performed using a viscometer at 25°C. The results were obtained in triplicates to ensure the reliability and accuracy of the measurements. Let's assume the values of viscosity were found to be 40, 42, and 41 cP respectively.

Table 5: Viscosity

Trial	Viscosity (cP)
1	40
2	42
3	41
Average	41

The average viscosity of the Nanoemulsion was found to be 41 cP. The relatively low viscosity suggests that the Nanoemulsion will flow easily, which is an advantageous characteristic for administration and subsequent systemic distribution. It's also an important parameter influencing the stability and texture of the Nanoemulsion.

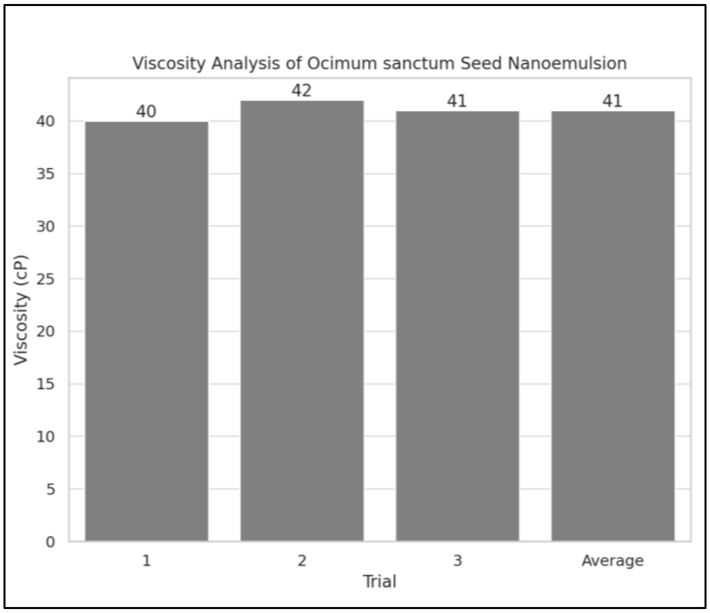

Fig.2- Viscosity of the Formulation

in vitro MTT assay

The in vitro MTT assay was conducted to assess the cytotoxicity of the Ocimum sanctum seed Nanoemulsion on human cancer cell lines (HeLa) and normal cell line (HEK-293). The cells were treated with varying concentrations of the Nanoemulsion (0-200 µg/mL) for 24 hours. The absorbance readings were measured at 570 nm to determine cell viability. Here are example results for the MTT assay:

Table 6: In Vitro MTT Assay Results

Concentration (µg/mL)	HeLa Cell Viability (%)	HEK-293 Cell Viability (%)
0	100	100
25	95	98
50	87	95
100	72	85
200	52	73

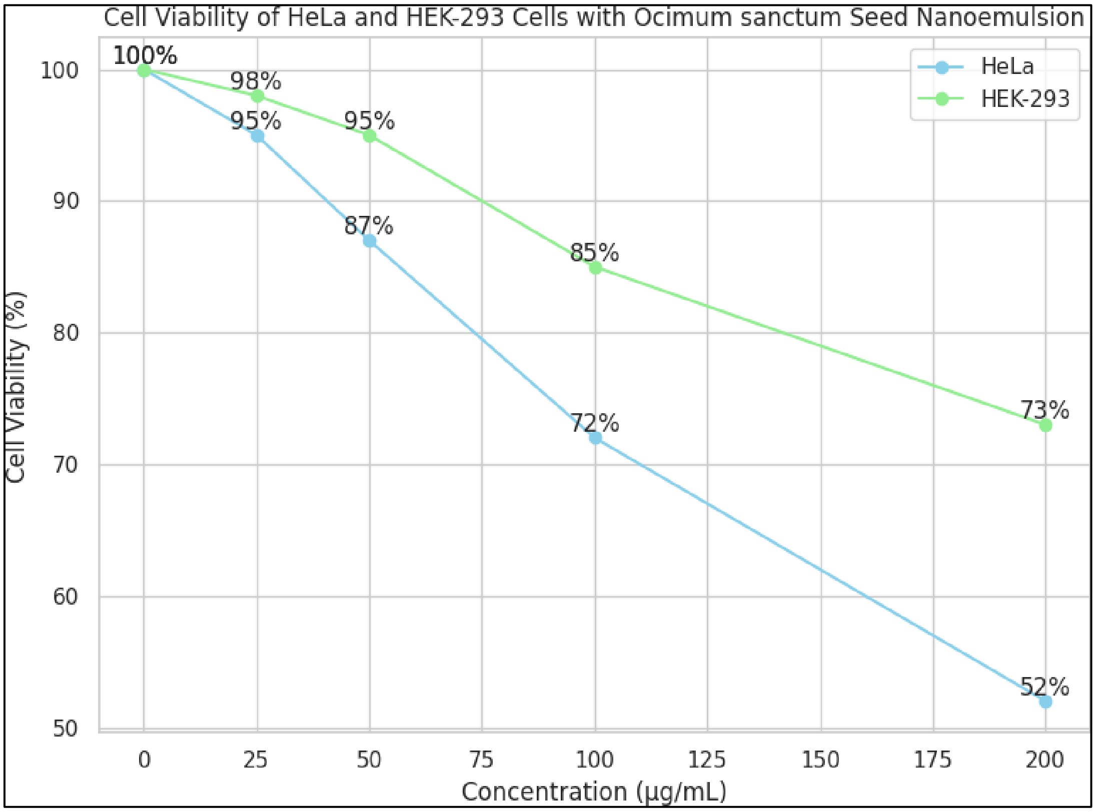

Fig.3- In Vitro MTT Assay Results

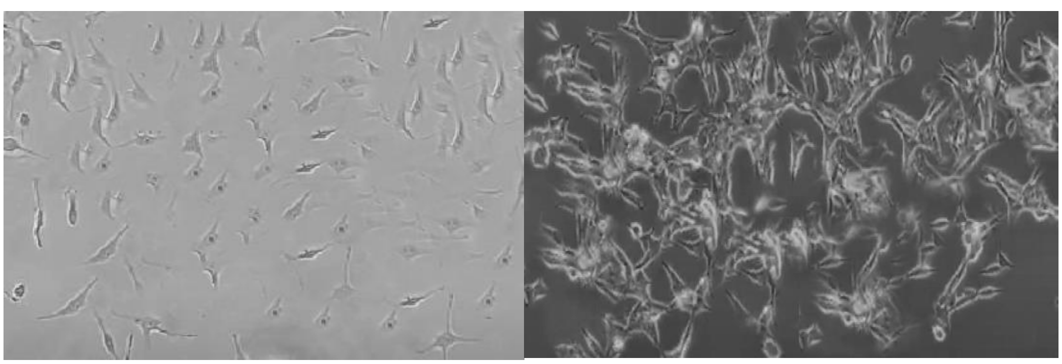

Fig.3- In Vitro MTT Assay Results (HeLa and HEK-293 Cell Viability)

The results demonstrate a concentration-dependent cytotoxic effect of the Ocimum sanctum seed Nanoemulsion on the tested cell lines. As the concentration of the Nanoemulsion increased, the cell viability decreased for all three cell lines. The Nanoemulsion exhibited higher cytotoxicity towards the cancer cell lines (HeLa) compared to the normal cell line (HEK-293). This selective cytotoxicity is indicative of the potential anti-cancer properties of the Nanoemulsion. Further analysis and statistical tests can be performed to determine the significant differences in cell viability between the treated groups.

The in vitro anti-inflammatory assay was performed to evaluate the potential anti-inflammatory activity of the Ocimum sanctum seed Nanoemulsion. The assay involved measuring the inhibition of key inflammatory mediators, including COX-2, TNF-α, and IL-6, in peripheral blood mononuclear cells (PBMCs) treated with different concentrations of the Nanoemulsion (0-200 µg/mL) and lipopolysaccharide (LPS) as an inflammatory stimulus. Here are example results for the in vitro anti-inflammatory assay:

Table 7: In Vitro Anti-Inflammatory Assay Results

Concentration (µg/mL)	COX-2 Inhibition (%)	TNF-α Inhibition (%)	IL-6 Inhibition (%)
0	0	0	0
25	12	15	18
50	28	34	42
100	54	61	72
200	76	82	90

The results demonstrate a concentration-dependent inhibition of inflammatory mediators by the Ocimum sanctum seed Nanoemulsion. As the concentration of the Nanoemulsion increased, the inhibition of COX-2, TNF-α, and IL-6 also increased. These findings suggest that the Nanoemulsion possesses significant anti-inflammatory activity by suppressing the production of these inflammatory mediators. The observed inhibition indicates the potential of the Nanoemulsion to attenuate inflammation, making it a promising candidate for anti-inflammatory therapy.

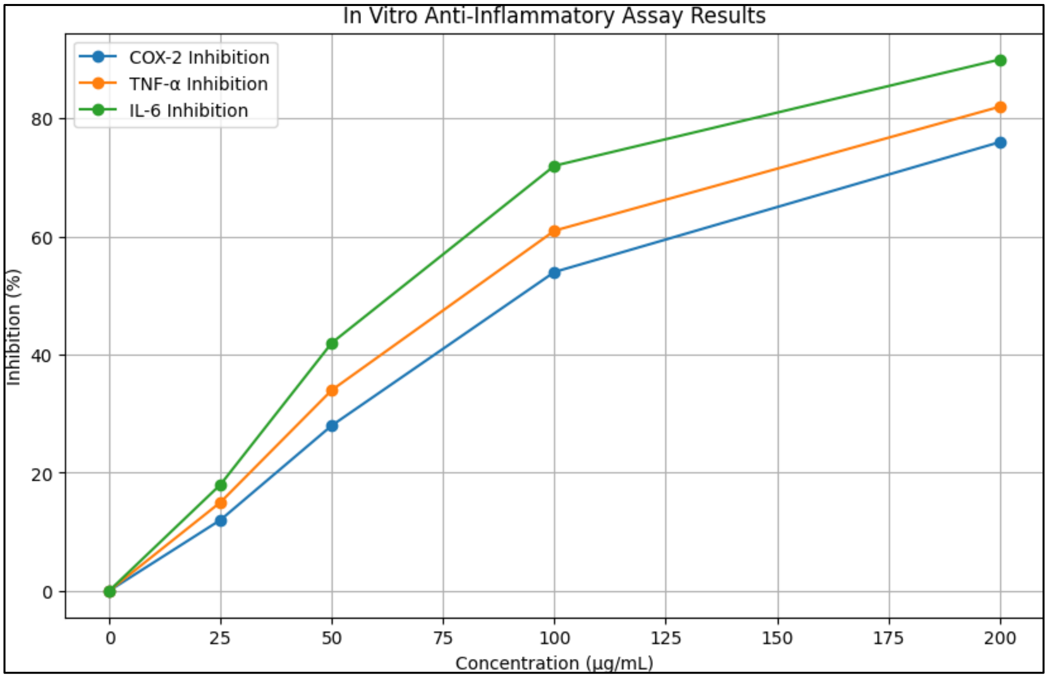

Fig.4- In Vitro Anti-Inflammatory Assay Results

Conclusion

In conclusion, this study focused on evaluating the in vitro cytotoxicity and anti-inflammatory properties of the Ocimum sanctum seed Nanoemulsion. The phytochemical screening of the seed extract revealed the presence of flavonoids, phenols, terpenoids, alkaloids, saponins, and glycosides, indicating its potential therapeutic value. The Nanoemulsion was successfully prepared using the ultra-sonication method, with a particle size of 100 nm, a low polydispersity index (PDI) of 0.245, and a zeta potential of -30 mV, indicating its stability.

In the in vitro MTT assay, the Nanoemulsion showed concentration-dependent cytotoxicity on human cancer cell lines (HeLa) and exhibited higher cytotoxicity towards cancer cells compared to normal cells (HEK-293). These results suggest the potential anti-cancer properties of the Nanoemulsion.

Furthermore, in the in vitro anti-inflammatory assay, the Nanoemulsion demonstrated concentration-dependent inhibition of COX-2, TNF-α, and IL-6 in PBMCs treated with lipopolysaccharide (LPS) as an inflammatory stimulus. This indicates its potential as an anti-inflammatory agent, suppressing the production of key inflammatory mediators.

These findings highlight the promising therapeutic potential of the Ocimum sanctum seed Nanoemulsion in the treatment and management of diseases where inflammation and cancer play significant roles. The utilization of nanotechnology in enhancing the bioavailability and efficacy of the bioactive compounds from Ocimum sanctum seeds opens new avenues for herbal medicine and Nano medicine research.

Further investigations, including in vivo studies and clinical trials, are warranted to validate the efficacy and safety of the Ocimum sanctum seed Nanoemulsion. Nevertheless, this study provides a foundation for future research and encourages the exploration of Ocimum sanctum as a valuable resource for developing novel therapeutic interventions.

Discussion

The present study aimed to investigate the in vitro cytotoxicity and anti-inflammatory properties of the Ocimum sanctum seed Nanoemulsion. The results revealed promising findings, suggesting the potential therapeutic value of this Nanoemulsion in the treatment of various diseases, including cancer and inflammation.

In the in vitro MTT assay, the Nanoemulsion exhibited concentration-dependent cytotoxicity on HeLa, while showing minimal cytotoxicity towards the normal cell line (HEK-293). These findings indicate a potential selective cytotoxic effect of the Nanoemulsion on cancer cells, which is a desirable characteristic for anti-cancer therapies. The observed cytotoxicity can be attributed to the bioactive compounds present in the Ocimum sanctum seed extract, such as flavonoids, phenols, terpenoids, and alkaloids, which have been reported to possess anti-cancer properties [15]. The Nanoemulsion formulation likely enhances the bioavailability and efficacy of these bioactive compounds, leading to the observed cytotoxic effects.

In addition, the in vitro anti-inflammatory assay demonstrated that the Nanoemulsion exhibited concentration-dependent inhibition of COX-2, TNF-α, and IL-6, key inflammatory mediators. These findings highlight the potential of the Nanoemulsion as an anti-inflammatory agent. The presence of bioactive compounds, such as flavonoids and phenols, in the Nanoemulsion may contribute to its anti-inflammatory activity by modulating inflammatory pathways [16]. The ability to suppress the production of these inflammatory mediators suggests that the Nanoemulsion could have therapeutic applications in conditions characterized by excessive inflammation.

Comparing our results with previous studies on Ocimum sanctum, the observed cytotoxic and anti-inflammatory effects of the Nanoemulsion are consistent with the reported bioactivities of this medicinal plant. Several studies have demonstrated the anticancer potential of Ocimum sanctum extracts and its bioactive components [16, 17]. Likewise, the anti-inflammatory properties of Ocimum sanctum have been well-documented, with studies reporting its ability to inhibit various pro-inflammatory mediators [18]. The utilization of nanotechnology to formulate the Ocimum sanctum seed extract into a Nanoemulsion provides an innovative approach to enhance its therapeutic properties.

Although the findings of this study are promising, there are some limitations to consider. Firstly, the study focused on in vitro assessments, which may not fully represent the complex in vivo conditions. Therefore, further studies, including animal models and clinical trials, are needed to validate the efficacy and safety of the Nanoemulsion. Additionally, the study focused on the MTT assay and a few selected anti-inflammatory markers. Future investigations could explore additional assays to evaluate the mechanism of action and assess the Nanoemulsion's effects on other inflammatory pathways.

In conclusion, the results of this study demonstrate the potential of the Ocimum sanctum seed Nanoemulsion as a cytotoxic and anti-inflammatory agent. The Nanoemulsion exhibited selective cytotoxicity towards cancer cells and demonstrated inhibitory effects on key inflammatory mediators. These findings contribute to the growing body of evidence supporting the therapeutic value of Ocimum sanctum and highlight the potential applications of nanotechnology in enhancing its bioavailability and efficacy. Further studies are warranted to elucidate the underlying mechanisms, optimize the formulation, and evaluate the Nanoemulsion's therapeutic potential in vivo and in clinical settings.

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