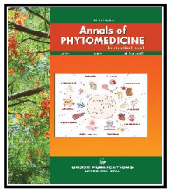
Annals of Phytomedicine 13(2): 544-558, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.2.55

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Hepatoprotective potential of curcumin loaded Avocado oil-based nanoemulsion system

Jyoti Kumari◆ and Anoop Kumar*

School of Medical and Allied Sciences, KRMU, Gurgaon-122103, Haryana, India
** Dr. KN Modi Institute of Pharmaceutical Research and Education, Modinagar-201204, Ghaziabad, Uttar Pradesh, India*

Article Info

Article history
Received 17 October 2024
Revised 5 December 2024
Accepted 6 December 2024
Published Online 30 December 2024

Keywords
Avocado oil
Nanoemulsion
Curcumin
Hepatoprotective effect
CCl_4-induced hepatotoxicity
Antioxidant activity
Liver protection

Abstract

Avocado oil (AO), an edible oil possesses enriched antioxidant activity and potential to modulate drug permeability. AO an advantageous base for a nanoemulsion delivery platform, especially for poorly water-soluble drugs or drugs with impaired permeability. Present study describes the development and AO-based curcumin nanoemulsion (NE) to address curcumin's poor bioavailability and assessment of hepatoprotective efficacy Optimized curcumin nanoemulsion formulation (F5), was characterized for pH, encapsulation efficiency and droplet size. Antioxidant capacity of (F5) was assessed using DPPH radical scavenging activity, demonstrated free radical neutralization. Assessment of hepatoprotective studies at low-dose (NELD: 10 mg/kg) and high-dose (NEHD: 20 mg/kg) nanoemulsion treatments against CCl_4-induced hepatotoxicity. NEHD significantly mitigated CCl_4-induced hepatotoxicity, demonstrated hepatoprotective effect amongst all groups. NEHD treatment led to a notable reduction in liver biomarkers and preserved liver histology, surpassing the efficacy of conventional curcumin doses. Histological analysis showed that inflammation and cellular damage observed in the NEHD were recovered and supports protective role against oxidative liver damage. These findings suggest that high-dose AO-based curcumin nanoemulsion enhances curcumin's bioavailability and therapeutic action, making it a promising candidate for liver protection. This study underscores the potential of nanoemulsion delivery systems in maximizing curcumin's clinical benefits for hepatoprotection.

1. Introduction

Drug delivery systems therapeutic effectiveness play a crucial role in the pharmaceuticals. They ensure that drugs reach their target sites in the body in the right concentration and at the appropriate time, optimizing the therapeutic outcome. Conventional formulations often face challenges like poor solubility, inadequate absorption and rapid metabolism all of which can hinder a drug's efficacy. To overcome these limitations, advanced drug delivery systems, have been developed to enhance the bioavailability of poorly soluble drugs (Bobade *et al*., 2016).

Self-nanoemulsifying drug delivery system (SNEDDS) is an advanced pharmaceutical approach designed to enhance the solubility and bioavailability of poorly water-soluble drugs. A SNEDDS formulation typically consists of a combination of oils, surfactants, and co-surfactants, which spontaneously form a nanoemulsion upon contact with gastrointestinal fluids. These emulsions, with droplet sizes typically less than 100 nanometres, significantly enhance drug absorption by increasing the surface area for solubilization and promoting gastrointestinal stability. The nanoscale dispersion improves the drug's dissolution and facilitates efficient absorption into the bloodstream (Ben-Shabat *et al*., 2020).

SNEDDS is particularly advantageous for addressing the limitations of conventional drug formulations, especially for drugs with poor water solubility and low bioavailability. By enhancing solubility and permeability, SNEDDS improves drug delivery efficiency. Additionally, its flexible formulation, ease of administration and potential for improving patient compliance make it a promising platform for a wide range of pharmaceutical applications. The present study aims to explore the potential of SNEDDS as a delivery system for curcumin and Avocado oil. Both compounds are known for their therapeutic properties but are hindered by poor water solubility and stability. This study seeks to optimize a SNEDDS formulation that maximizes the solubility, stability and bioavailability of these compounds, thereby enhancing their therapeutic efficacy and applicability (Ahmed *et al*., 2022; Khalil *et al*., 2022).

Avocado oil (*Persea americana*), is rich in beneficial fats and bioactive compounds. It is primarily composed of monounsaturated fatty acids, oleic acid (70%) (Lin *et al*., 2018; Afzal *et al*., 2022; Gokkaya *et al*., 2022). Additionally, Avocado oil contains polyunsaturated fats like linoleic acid, saturated fats such as palmitic acid, and a variety of phytochemicals, including sterols, carotenoids, tocopherols (vitamin E) and polyphenols. The pharmacological potential of Avocado oil spans various therapeutic areas. It has shown promise in promoting cardiovascular health by reducing cholesterol levels and improving lipid profiles. Its antioxidant and anti-inflammatory

Corresponding author: Mrs. Jyoti Kumari
School of Medical and Allied Sciences, KR Mangalam University Gurgaon, 122103, Haryana, India
E-mail: iimtjyoti@gmail.com
Tel.: +91-9557355269

effects make it beneficial for skin conditions like psoriasis and eczema. Moreover, Avocado oil is being studied for its role in enhancing wound healing and improving the absorption of fat-soluble nutrients. Its protective role against oxidative stress also suggests potential in cancer prevention (Afzal *et al*., 2022; Gokkaya *et al*., 2022).

Curcumin (*Curcuma longa*) is widely recognized for its potent therapeutic effects, including anti-inflammatory properties. However, its clinical application is limited by poor water solubility, rapid metabolism and low bioavailability (Ali *et al*., 2022). To address these challenges, advanced drug delivery systems such as nanoemulsions have been developed to enhance curcumin's therapeutic effectiveness. Avocado oil, rich in monounsaturated fats and bioactive compounds, serves as an ideal lipid-based carrier due to its potential to improve drug solubility and bioavailability. The selected doses of curcumin and the nanoemulsion formulation should be justified based on existing literature or preliminary studies. For instance, prior research has demonstrated that curcumin doses in the range of 100 to 500 mg/day are effective and safe in humans, with nanoemulsion-based delivery systems showing a marked improvement in bioavailability (Ali *et al*., 2022; Smith *et al.,* 2021). Additionally, Avocado oil has been shown to enhance lipid-soluble drug absorption, further supporting its inclusion in the formulation (Jurenka and Ascp, 2009; Clower *et al*., 2022; Gharat *et al*., 2024).

Based on the study, it is aimed to focuses on the development of an Avocado oil-based nanoemulsion system loaded with curcumin to overcome its bioavailability challenges. The novelty of this approach lies in the use of Avocado oil as the lipid phase, leveraging its beneficial chemical composition to stabilize the nanoemulsion, additive therapeutic effect and promote enhanced absorption of curcumin. This formulation could improve curcumin's therapeutic outcomes, particularly in treating inflammation-related conditions, while also offering the additional health benefits inherent to Avocado oil. By combining the pharmacological strengths of both curcumin and Avocado oil in a novel nanoemulsion system, this study aims to provide a promising, synergistic therapeutic strategy for enhancing curcumin's efficacy in clinical applications.

2. Materials and Methods

2.1 Chemical and reagents

Tween 80 was used as a surfactant, while PEG 400 acted as a co-surfactant, with curcumin also being utilized. Polyethylene glycol, PEG 200, and Tween 20 were obtained from Sisco Research Laboratories Pvt. Ltd. (SRL, Mumbai, India). HPLC-grade methanol was sourced from Merck Pvt. Ltd. (Mumbai). Loba Chemie Pvt. Ltd. (Mumbai, India) supplied HPLC-grade water, and Avocado oil was acquired from CDH (Delhi, India). All other chemicals, solvents, and reagents used in the study were of analytical grade.

2.2 UV Spectrophotometric calibration profiling

With minor modifications, the usual technique was followed in order to establish the maximal absorption of curcumin. In brief, the stock solution (1 mg/ml in methanol) was prepared and then its subsequent dilutions (0, 1, 2, 3, 4, and 5 µg/ml) were made and UV analysed at the 426 nm. The measurement was made three times, and statistical analysis was done on the collected data, as stated (Kumar *et al*., 2020).

2.3 Fourier transform infrared spectroscopy (FTIR)

A Parkin Elmer spectrophotometer was used to analyse the spectrum for SWM. According to Hosseini *et al*. (2019), each sample was separated using potassium bromide and then subjected to spectroscopic observation throughout the 4000 to 400 cm^{-1} range (Kumar *et al*., 2020).

2.4 Development of nanoemulsion

To prepare the SNEEDS formulation, Avocado oil was selected as the oil phase, with Tween 80 serving as the surfactant and PEG 400 as the co-surfactant. Various volume ratios of the oil, surfactant, and co-surfactant were tested, including 1:0, 1:0.5, 1:1, 2:1 and 3:1. These combinations were thoroughly blended to create mixtures of the oil phase and the surfactant/co-surfactant mix (S_{mix}) in the specified ratios. The resulting blends were assessed for clarity and then titrated with water drop by drop under gentle stirring, noting any changes in clarity or turbidity. Each formulation was prepared in triplicate for consistency.

A curcumin-loaded nanoemulsion (NE) system was prepared using an ultrasonication method. Initially, 1% w/w (10 mg/g) of curcumin was blended into the oil and S_{mix} phases to create an oil-in-water (O/W) dispersion using a vortex mixer. The aqueous phase was then gradually introduced while maintaining continuous vortexing for one minute. This mixture was subsequently processed with an ultrasonic homogenizer, applying ultrasonication in a water bath at 40% amplitude (120 W) for 3 to 5 min. Eight different NE formulations with varying component ratios were developed and evaluated for droplet size and polydispersity index (PDI) to identify the optimal formulation for curcumin encapsulation (Zakir *et al*., 2020).

2.4.1 Estimation of pH and viscosity of the developed nano-emulsion

The pH and viscosity of the curcumin-loaded nanoemulsion (NE) system were determined to evaluate its physicochemical properties. For pH measurement, a digital pH meter was used. A small amount of the NE sample was placed in the probe, and the pH value was recorded at room temperature (Dhama *et al*., 2022). For viscosity analysis, a Brookfield Viscometer was used. The NE sample was placed in the viscometer and measurements were taken at different shear rates to determine its viscosity. These assessments provided important information on the NE system's suitability for application and stability under varying conditions (Ahmad *et al*., 2014).

2.4.2 Thermodynamic stability

The thermodynamic stability of the nanoemulsion system was assessed under different stress conditions. Initially, the system was subjected to heating and cooling cycles, alternating between 4°C and 40°C, with samples maintained at each temperature for 48 h. Subsequently, freeze-thaw cycles were conducted, where the samples were stored at – 21°C and +25°C for 48 h each. These tests were conducted to assess the NE system's ability to maintain stability under fluctuating temperatures (Kumar *et al*., 2023).

In addition, a centrifugation stress test was performed. For this, 1ml of the curcumin-loaded NE was diluted to 100 ml with distilled water, and the solution was subjected to centrifugation at 5000 rpm for 30 min. The samples were visually inspected after centrifugation to check for any signs of phase separation, which would indicate instability. These tests helped confirm the robustness and stability of the curcumin-loaded NE system under varying conditions (Hussain *et al*., 2014).

2.4.3 Analysis for curcumin encapsulation

The curcumin content in the prepared nanoemulsion (NE) formulations was quantified using a UV-visible spectrophotometer set to a wavelength (λ_{max}) of 426 nm. To determine the concentration accurately, a sample preparation process was conducted. Specifically, a 100 µl sample of the curcumin-loaded NE was taken and diluted with 100 ml of methanol. The mixture was vortexed thoroughly to dissolve the curcumin completely, forming a uniform solution. This prepared sample was then analyzed at 426 nm using the spectrophotometer. A standard calibration curve of known curcumin concentrations was employed to ensure precise and accurate quantification of the curcumin content in the NE formulations (Dhanasekaran *et al*., 2018).

2.4.4 Size distribution analysis

The average droplet size, polydispersity index (PDI), and zeta potential of the curcumin-loaded nanoemulsion (NE) formulations were evaluated using the Dynamic light scattering (DLS) method. Measurements were conducted at 25°C using the Zetasizer nano ZS90 (Malvern Instruments, Malvern, UK). For droplet size and PDI determination, a small amount of the NE sample was diluted with deionized water to minimize multiple scattering effects. The diluted sample was placed in a disposable cuvette, and measurements were performed at a scattering angle of 90°C. The DLS technique monitored fluctuations in the intensity of scattered light caused by the brownian motion of the droplets. Data analysis was carried out using the cumulant method to calculate the average droplet size (z-average) and the PDI, which reflects the width of the particle size distribution. The results were also analyzed for number-based size distributions to provide additional insights into the particle population.

The zeta potential, an indicator of surface charge and colloidal stability, was measured using the same instrument with disposable folded capillary cells. This measurement involved applying an electric field and analyzing the electrophoretic mobility of the particles to determine their surface charge. Together, these measurements provided critical information about the size distribution, uniformity, surface charge, and stability of the curcumin-loaded NE formulations, contributing to the evaluation of their quality and performance (Duarte *et al*., 2023).

In the evaluation of nanoemulsion formulations using Avocado oil, Tween 80, and PEG 400, the formulations were analyzed to identify the optimal composition based on particle size, polydispersity index (PDI), and intercept values. Among the five formulations (F1 to F5), the best results were observed with formulation F5, which used a S_{mix} ratio of 3:1 (Tween 80: PEG 400). F5 showed a particle size of 16.67 ± 0.99 nm, the smallest among all formulations, indicating a highly stable nanoemulsion with better dispersion. Additionally, F5 exhibited a PDI of 0.424 ± 0.009, suggesting a narrow size distribution and uniformity in particle size. The intercept value for F5 was 0.798 ± 0.008, further supporting its stability and effectiveness compared to other formulations.

In contrast, formulations like F3 had much larger particle sizes (1040.0 ± 34.97 nm) and higher PDI values, indicating poor stability and a less favorable emulsion. The S_{mix} ratio of 3:1 in F5 likely provided an ideal balance between surfactant and co-surfactant, effectively reducing the interfacial tension and producing smaller droplets, resulting in a stable and efficient nanoemulsion (Crucho and Barros, 2017).

Table 1: Formulation of Avocado oil based nanoemulsion

Formulation code	Avocado oil	Tween 80 (%)	PEG 400(%)	S_{mix} ratio	Size	PDI	Intercept	Interference
F1	10 ml	5	0	1: 0	193.23 ± 6.38	1.354 ± 0.02	0.372 ± 0.004	TURBID
F2	10 ml	5	2.5	1: 0.5	83.92 ± 4.29	0.521 ± 0.009	0.713 ± 0.003	CLEAR
F3	10 ml	5	5	1: 1	1040.0 ± 34.97	1.000 ± 0.04	0.788 ± 0.01	CLEAR
F4	10 ml	10	5	2: 1	27.09 ± 2.27	0.423 ± 0.02	0.783 ± 0.009	CLEAR
F5	10 ml	15	5	3: 1	16.67 ± 0.99	0.424 ± 0.009	0.798 ± 0.008	CLEAR

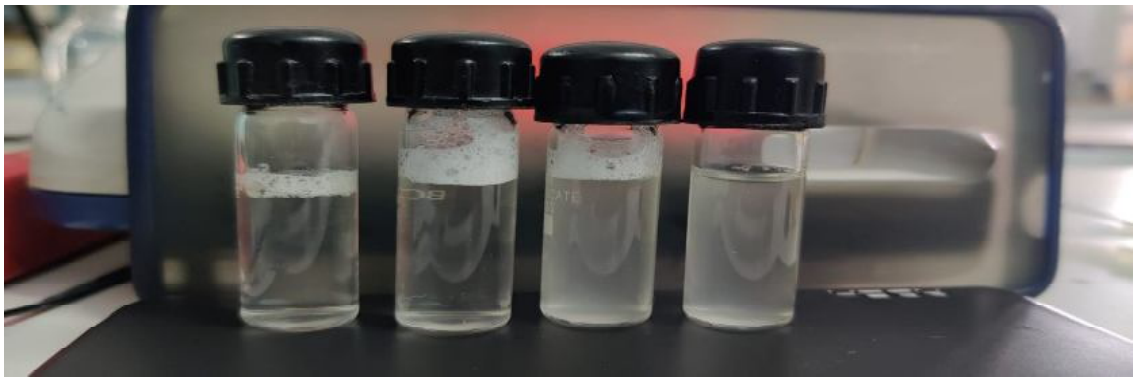


Figure 1: Nanoemulsion formulation of Avocado oil.

2.5 *In vitro* DPPH antioxidant activity of optimized nanoemulsion

This study aimed to assess the antioxidant activity of a formulated and optimized nanoemulsion (F5), in relation to the oil used and with ascorbic acid serving as the standard reference drug. The procedure, adapted with modifications from the protocol outlined by Gaurav and his team involved the preparation of a 1 mg/ml stock solution of both the sample and the standard drug. Subsequently, various concentrations 10, 20, and 30 µg/ml were prepared. To test the scavenging effect against DPPH free radicals (0.01 mM solution in methanol), 20 µl of the sample was combined with 180 µl of the DPPH solution in a 96-well ELISA plate. The plate was then incubated in the dark for 30 min. afterward, the absorbance of each sample and standard was measured spectrophotometrically at 517 nm. To determine the antioxidant activity, the following equation was used (Gaurav *et al*., 2020):

$$\text{Percentage scavenging activity} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

*A= absorbance

2.6 *In vivo* hepatoprotective activity

2.6.1 Experimental animals

Under the ethical approval number MIET/IAEC/CPCSEA/2023/91, the *in vivo* experimental tests were carried out using Wistar albino rats weighing 150 to 200 g. The animals were kept in cages made of polypropylene and acclimated to the controlled circumstances of the laboratory, which included a 12 h light/dark cycle, a temperature of 23 ± 2°C and a relative humidity of 55 ± 5%. The rats were fed a typical pellet meal and had unlimited access to regular saline throughout the research. Following the rules set forth by the institutional animal ethical committee (IAEC) in India and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), the study closely complied with ethical standards (Evan Prince, 2018).

2.6.2 Treatment schedule

Thirty-six rats were divided into six groups, each with six individuals, at random for this investigation. For seven days, 0.1 ml of a 0.5% carboxymethyl cellulose (CMC) solution was administered orally to the control group (Group 1). Likewise, CMC was given to Group 2 (CCl_4 group). Group 3 was given oral silymarin once day at a dose of 25 mg/kg. A nanoemulsion formulation was administered to groups 4 and 5 at low dosages (10 mg/kg) and high doses (20 mg/kg), respectively. Curcumin was administered to groups 6 and 7 at low dosages (50 mg/kg) and high doses (100 mg/kg). Rats in Groups 2 to 7 got intraperitoneal injections of 0.5 ml/kg of CCl_4 on the seventh day, whereas Group 1 was given an equivalent volume of regular saline. Following an overnight fast, all rats had their blood drawn on the seventh day *via* retro-orbital puncture while under light ether anesthesia. The rats were put to death by cervical dislocation while under light ether anesthesia, and the serum was isolated for biochemical examination. After that, liver tissues were removed and kept for later analysis at –80°C (Jain *et al*., 2012).

2.6.2.1 Biochemical analysis

Serum samples drawn from the blood of rats in different experimental groups were subjected to biochemical analysis in order to assess markers of liver function. Alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total protein levels were among them. The assessments were performed in accordance with the standard procedures specified by Reckon Diagnostics, Baroda, India, ensuring precision and consistency in the measurements of these liver function parameters (Velraj *et al*., 2024). The treated rats' liver tissues were carefully removed and homogenized at a 10% (w/v) dosage in cold 10 mM Tris buffer (pH 7.4). A high-speed chilled centrifuge was then used to centrifuge the homogenized samples for 20 min at 4°C at 10,000 × g. The levels of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) were measured using biochemical tests on the resultant supernatants. Every assay was carried out strictly in accordance with the guidelines supplied by the individual kit makers (Khan *et al*., 2024).

2.6.2.2 Histopathological analysis

Liver tissues were first preserved in a 10% formalin solution for histological analysis of the liver in both control and treatment animals. After fixation, the tissue samples were embedded in paraffin wax after being dehydrated using a series of graded alcohol solutions. To see the overall tissue structure, thin sections that were roughly 5 µm thick were cut using a microtome and stained with hematoxylin and eosin (H&E). After that, the dyed slides were examined under a light microscope to assess any histopathological abnormalities, such as fibrosis, necrosis, inflammation and other cellular changes (Schmidt *et al*., 2007).

2.7 Statistical analysis

Two-way and one-way ANOVA were used in the statistical analysis to evaluate the variations in means among the different groups. Statistical significance was defined as a *p*-value over 0.05. Tukey's test was used to compare the groups pairwise. The mean ± standard deviation (SD) is used to display the results. The results were summarized, and significant differences between treated and control groups were highlighted. The *p*-values from ANOVA tests determined whether the variations observed across groups were statistically significant.

3. Results

3.1 UV Spectrophotometric calibration profiling

The method development and calibration analysis of curcumin was successfully performed. Each measurement was conducted in triplicate and statistically represented. The developed method demonstrated linearity, precision, and accuracy across curcumin concentrations ranging from 1 to 5 µg/ml in each dilution. The calibration equation for the method was determined to be $y = 0.1653x - 0.0051$, with a regression coefficient (R^2) of 0.9981, indicating excellent linearity. The limit of detection (LOD) was calculated as 0.0779 ± 0.0013 µg/ml, while the limit of quantitation (LOQ) was determined to be 0.2420 ± 0.0018 µg/ml.

Further elaboration of the FTIR spectra interpretation revealed specific functional groups responsible for curcumin's properties and its interactions with the nanoemulsion components. These findings

highlight the significant roles of the functional groups in facilitating the stability and efficacy of curcumin within the nanoemulsion system. The results of this study, including the calibration curve and FTIR spectral analysis, are visually represented in Figure 2.

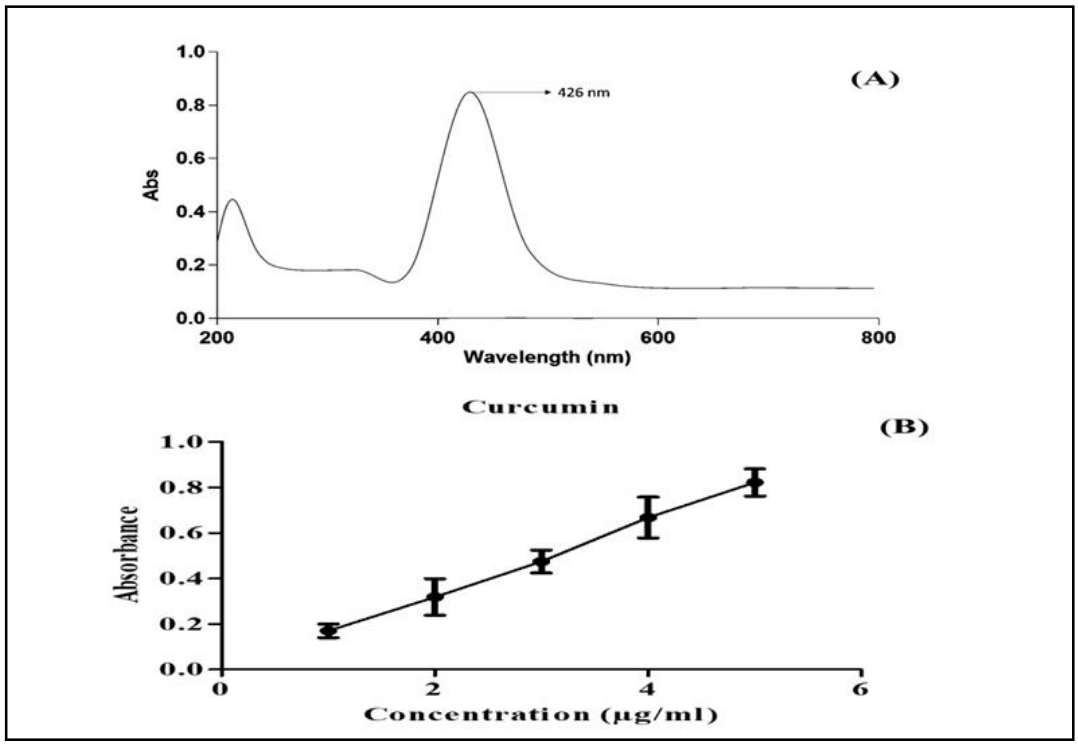


Figure 2: Curcumin developed spectrum (Figure A) and calibration curve developed against different concentrations (Figure B).

3.2 Fourier transform infrared spectroscopy (FTIR)

A Parkin Elmer spectrophotometer was used to analyse the spectrum for SWM and spectroscopic observation were made throughout the 400 to 400 cm^{-}1 range. In this analysis, curcumin and FTIR spectrum were developed and characterised based on their peak intensity and ranging of the functional group. The results of FTIR spectrum for curcumin shows characteristic peaks that indicate the presence of key functional groups. A broad absorption band around 3500 cm^{-1} corresponds to O-H stretching vibrations, likely due to hydroxyl groups in the structure of curcumin. The peak near 2900 cm^{-1} can be attributed to C-H stretching, indicative of alkyl groups. A strong absorption around 1625 cm^{-1} represents the C=O stretching vibration, corresponding to the carbonyl groups present in curcumin. The peaks in the region around 1508 cm^{-1} likely indicate C=C stretching vibrations from the aromatic rings. Additionally, the bands near 1000 to 1300 cm^{-1} are suggestive of C-O stretching vibrations, possibly from methoxy and hydroxyl groups. The presence of hydroxyl, carbonyl, and aromatic ring structures represents the essential components of curcumin. The FTIR spectrum of curcumin has been depicted in Figure 3.

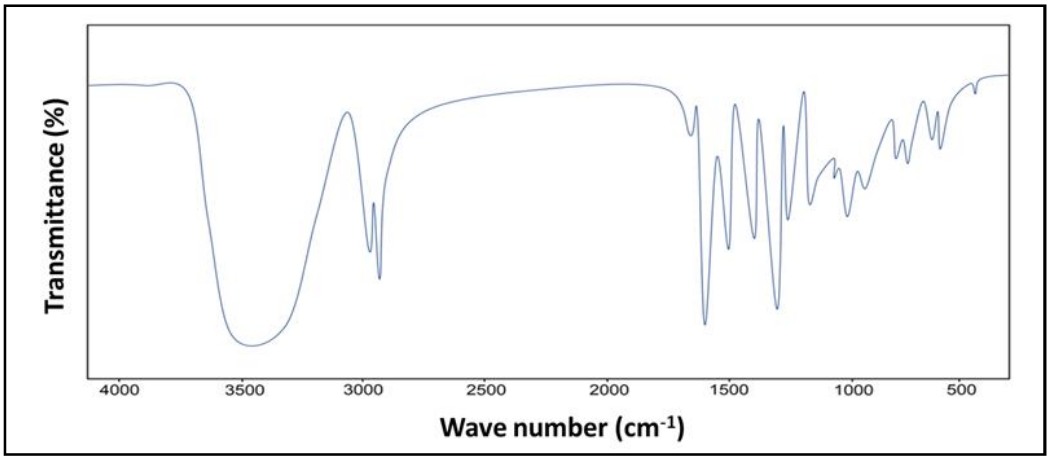


Figure 3: FTIR spectrum of curcumin showed several characteristic peaks at different regions.

Pandit and his team reported FTIR analysis of curcumin nanoparticles (nanocurcumin) revealed key functional groups that confirmed successful synthesis and structural integrity of curcumin. Characteristic peaks for hydroxyl (-OH) and carbonyl (C=O) groups were evident, suggesting that nanocurcumin retained curcumin's original chemical framework. These findings support nanocurcumin's stability, enhancing its solubility and antibacterial effectiveness (Pandit *et al*., 2015).

3.3 Development and optimization of nanoemulsion

The preparation of self-nanoemulsifying drug delivery systems (SNEDDS) utilized avocado oil as the oil phase, Tween 80 as the surfactant, and PEG 400 as the co-surfactant, with varying S_{mix} ratios (1:0, 1:0.5, 1:1, 2:1 and 3:1). The findings indicated that all the prepared nanoemulsions were clear, with the exception of the formulation with a S_{mix} ratio of 1:0. The curcumin-loaded nanoemulsion (NE) system ultrasonication provides the optimal dispersion of nanoparticles that provides as well as enhance its strength and stability. A 1% w/w concentration of curcumin was initially combined with the oil phase and S_{mix} using a vortex mixer. The aqueous phase was then added, and the resulting mixture was sonicated at 40% amplitude (120 W) for 3 to 5 min. Five distinct NE formulations were created and assessed for droplet size, polydispersity index (PDI), intercept, and interference. The formulation with the smallest droplet size and lowest PDI values was considered optimal, indicating enhanced stability and uniformity. Among the five formulations (F1 to F5), the best results were observed with formulation F5, which used a S_{mix} ratio of 3:1 (Tween 80: PEG 400).

F5 showed a particle size of 16.67 ± 0.99 nm, the smallest among all formulations, indicating a highly stable nanoemulsion with better dispersion. Additionally, F5 exhibited a PDI of 0.424 ± 0.009, suggesting a narrow size distribution and uniformity in particle size. The intercept value for F5 was 0.798 ± 0.008, further supporting its stability and effectiveness compared to other formulations.

In contrast, formulations like F3 had much larger particle sizes (1040.0 ± 34.97 nm) and higher PDI values, indicating poor stability and a less favorable emulsion. The S_{mix} ratio of 3:1 in F5 likely provided an ideal balance between surfactant and co-surfactant, effectively reducing the interfacial tension and producing smaller droplets, resulting in a stable and efficient nanoemulsion. The outcome of the study has been represented in the Table 2.

Table 2: Formulation of Avocado oil based nanoemulsion

Formulation code	Avocado oil	Tween 80 (%)	PEG 400 (%)	S_{mix} ratio	Size	PDI	Intercept	Interference
F1	10 ml	5	0	1: 0	193.23 ± 6.38	1.354 ± 0.02	0.372 ± 0.004	TURBID
F2	10 ml	5	2.5	1: 0.5	83.92 ± 4.29	0.521 ± 0.009	0.713 ± 0.003	CLEAR
F3	10 ml	5	5	1: 1	1040.0 ± 34.97	1.000 ± 0.04	0.788 ± 0.01	CLEAR
F4	10 ml	10	5	2: 1	27.09 ± 2.27	0.423 ± 0.02	0.783 ± 0.009	CLEAR
F5	10 ml	15	5	3: 1	16.67 ± 0.99	0.424 ± 0.009	0.798 ± 0.008	CLEAR

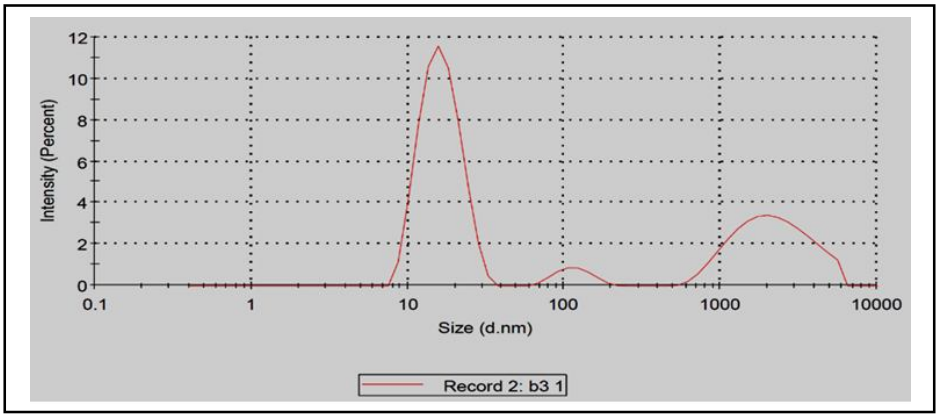


Figure 4: Size distribution analysis of optimized nanoemulsion formulation (F5: S_{mix} 3:1).

In a study by Algahtani and his team, a curcumin-loaded nanoemulsion was prepared using ultrasonic emulsification, resulting in droplets with an average size of 56.25 ± 0.69 nm and a PDI of 0.05 ± 0.01. When incorporated into a 0.5% Carbopol® 940 hydrogel, the formulation significantly enhanced the skin penetration and therapeutic effectiveness of curcumin. This was confirmed through *in vivo* wound healing experiments conducted on Wistar rats (Algahtani *et al*., 2021). Sheng and her team found that a turmeric extract (TUR), consisting of 59% curcumin, 22% demethoxy curcumin, and 18% bisdemethoxycurcumin, significantly improved the stability and antidepressant effects of curcumin *in vitro*. The study aimed to create a nano-delivery system (TUR-NE) to enhance the pharmacokinetics and antidepressant effectiveness of TUR. The TUR-NE formulation showed a particle size of 116.0 ± 0.31 nm, a PDI of 0.121 ± 0.007, and an encapsulation efficiency of 98.45%, indicating strong release characteristics and stability. Pharmacokinetic studies revealed that TUR-NE had substantially higher AUC (0-t) and C_{max} values compared to curcumin alone, indicating improved

bioavailability. Additionally, TUR-NE showed greater antidepressant efficacy in the chronic unpredictable mild stress (CUMS) model, elevating serotonin levels in both plasma and brain tissue. The enhanced antidepressant activity and safety profile of TUR-NE indicate that it could be a promising and effective oral treatment for depression, offering notable benefits compared to traditional curcumin formulations (Sheng *et al*., 2023).

Fatease and his team 2023 developed a biocurcumin nanoemulsion (CMN-NEs) for transdermal administration to enhance treatment. Using a self-nanoemulsification approach, the nanoemulsion was formulated with Cremophor EL, glyceryl monooleate, and PEG 5000. The resulting nanoemulsion exhibited favorable properties, including a droplet size of 90.0 ± 2.1 nm, a zeta potential of – 7.4 ± 0.4 mV, and a PDI of 0.171 ± 0.03. The nanoemulsion significantly increased curcumin's permeability and stability, showing improved bioavailability and better skin penetration. Additionally, the CMN-NE gel demonstrated enhanced antifungal efficacy against *Candida albicans* compared to conventional treatments (Al Fatease *et al*., 2023).

The study demonstrated the potential of a nanoemulgel formulation combining curcumin (CUR) and cyclosporine (CYC) for effective rheumatoid arthritis (RA) management. The nanoemulsion, prepared using spontaneous emulsification, was incorporated into a Carbopol® gel, resulting in a small globule size (15.32 nm), favorable polydispersity index (0.181), and stable zeta potential (-16.3 mV). The formulation exhibited excellent drug release profiles, with nearly complete CUR and CYC release in 24 h. *In vitro* experiments showed notable decreases in pro-inflammatory cytokines (IL-6, TNF-α) and prostaglandin E2, alongside an increase in the anti-inflammatory cytokine IL-10 levels. The nanoemulgel also showed promising antiarthritic effects in an animal model, reducing symptoms and inflammation (Gharat *et al*., 2024).

3.4 Evaluation of nanoemulsion

3.4.1 Estimation of pH and viscosity of the developed nanoemulsion

The study revealed that the curcumin-loaded optimized nanoemulsion (NE) system exhibited a pH value of 6.3 ± 0.31, which is suitable for topical applications, ensuring minimal skin irritation and compatibility with physiological pH. Additionally, the viscosity of the NE was measured at 2733 ± 71.29 Cps, indicating a stable formulation with appropriate flow characteristics for efficient application. The moderate viscosity suggests ease of spreading on the skin, while maintaining the structural integrity of the nanoemulsion. These results demonstrate the optimized NE (F5) potential for effective topical delivery, balancing both stability and user-friendliness.

3.4.2 Thermodynamic stability

Thermodynamic stability is crucial for nanoemulsions, as it provides a longer shelf life compared to ordinary emulsions that only have kinetic stability. This stability ensures that nanoemulsions remain uniformly dispersed without phase separation, which is common in kinetically stable systems. As a result, thermodynamically stable nanoemulsions offer enhanced durability and are preferable for applications requiring prolonged stability (Azeem *et al*., 2009). In this study, the thermodynamic stability of the curcumin-loaded nanoemulsion (NE) system was assessed through a series of stress tests. The findings showed that the NE system maintained outstanding stability under various conditions. No phase separation, precipitation, or cloudiness was observed during heating–cooling cycles (ranging from 4°C to 40°C) or freeze–thaw cycles (from 21°C to +25°C), suggesting the formulation remained stable even with temperature variations. This highlights the robustness of the curcumin-loaded NE for use in varying environmental conditions, which is crucial for maintaining product quality during storage and transport.

In the centrifugation stress test, after dilution of the NE (1 ml in 100 ml distilled water) and centrifugation at 5000 rpm for 30 min, the formulation showed no signs of phase separation. This further confirmed the NE's stability and ability to withstand mechanical stress without compromising its structure. Moreover, the optimized formulation (F5), with a Smix ratio of 3:1 (Tween 80: PEG 400), demonstrated the smallest particle size (16.67 ± 0.99 nm) and a low polydispersity index (PDI) of 0.424 ± 0.009, indicating a stable and uniform emulsion. The NE also showed a clear appearance, reinforcing the stability of the system. In contrast, other formulations such as F1 and F3 exhibited larger particle sizes and higher PDI values, resulting in reduced stability and turbid appearances. The outcome of thermodynamic stability has been represented in the Table 4.

Table 3: pH and viscosity profiling of developed nanoemulsion

Formulation code	S_{mix} ratio	pH	Viscosity (Cps)
F1	1:0	7.12 ± 0.73	2899 ± 82.73
F2	1:0.5	7.01 ± 0.48	3013 ± 75.28
F3	1:1	6.69 ± 0.55	2965 ± 92.63
F4	2:1	6.38 ± 0.28	2725 ± 65.23
F5	3:1	6.32 ± 0.31	2733 ± 71.29

Table 4: Thermodynamic stability and characterization

Formulation code	S_{mix} ratio	Heating-cooling stability	Freeze-thaw stability	Centrifugation stability
F1	1:0	Unstable	Unstable	Unstable
F2	1:0.5	Unstable	Unstable	Unstable
F3	1:1	Stable	Unstable	Unstable
F4	2:1	Stable	Stable	Stable
F5	3:1	Stable	Stable	Stable

3.4.3 Analysis for curcumin encapsulation

This study specifically focusing on curcumin encapsulation or drug content across various formulations. The results show a progressive increase in curcumin encapsulation efficiency across formulations, with F1 demonstrating the lowest encapsulation at 64.673 ± 2.672 %, while F5 exhibits the highest encapsulation efficiency that was found as 96.273 ± 3.550 % formulation F2 shows a moderate increase in encapsulation efficiency, followed by F3 and F4, which depict further improvements. These results suggest that the formulation method significantly impacts the encapsulation efficiency, likely due to variations in surfactant concentration, oil phase, and co-surfactant ratios. The outcome of the study has been represented in Figure 5.

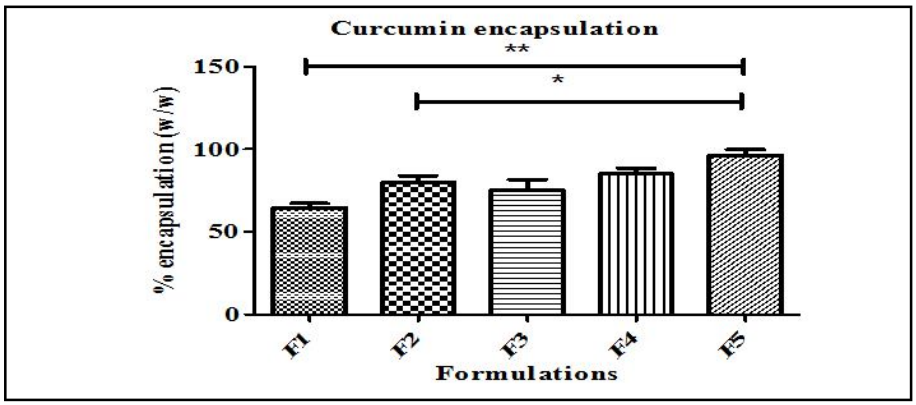


Figure 5: Curcumin nanoemulsion based encapsulated strength of different of nanoemulsion.

The increasing encapsulation efficiency observed from F1 to F5 suggests that adjusting the S_{mix} ratio (surfactant to co-surfactant mixture) plays a crucial role in improving curcumin loading in the nanoemulsion system. Formulation F5, with the highest encapsulation efficiency, likely benefits from an optimal S_{mix} ratio (3:1), as indicated by the smaller droplet size, better stability, and enhanced drug solubility. The reduced particle size in F5 ensures better surface area for curcumin encapsulation, improving drug loading and stability. In contrast, the lower encapsulation efficiency in F1 could be due to suboptimal surfactant levels, resulting in less efficient droplet formation and curcumin dispersion. As the formulations progress, higher surfactant levels and better emulsification likely contribute to the improved encapsulation seen in formulations F2 through F4. The study indicates that optimizing surfactant and co-surfactant concentrations in nanoemulsion formulations is critical for maximizing curcumin encapsulation, with F5 providing the most promising results for potential drug delivery applications.

3.4.4 FTIR spectroscopy of the developed and optimized nanoemulsion

In this study, the FTIR spectroscopy analysis was conducted of the developed and optimized nanoemulsion (F5) via correlation with blank formulation and curcumin. The pure curcumin spectrum (yellow) displays characteristic peaks at 3500 to 3200 cm^{-1} (O-H stretching vibrations), indicating phenolic hydroxyl groups. The peak around 1627 cm^{-1} corresponds to C=O stretching (keto group), and peaks near 1508 cm^{-1} and 1430 cm^{-1} represent the C=C aromatic stretching. Additionally, there is a sharp peak at 1270 cm^{-1}, which can be attributed to C-O stretching. In the blank spectrum (blue), there are no distinct peaks representing the curcumin's functional groups, as it primarily shows the presence of excipients used in the formulation matrix. This lack of curcumin-related peaks confirms the absence of the drug. For the optimized formulation (F5, orange), the characteristic peaks of curcumin, broadening of the O-H stretching band at 3200 to 3500 cm^{-1} and the characteristic peak of functional group such as C=O and C=C within range and least even no interaction of drug and excipient. The outcome of the spectral study is depicted in the Figure 6.

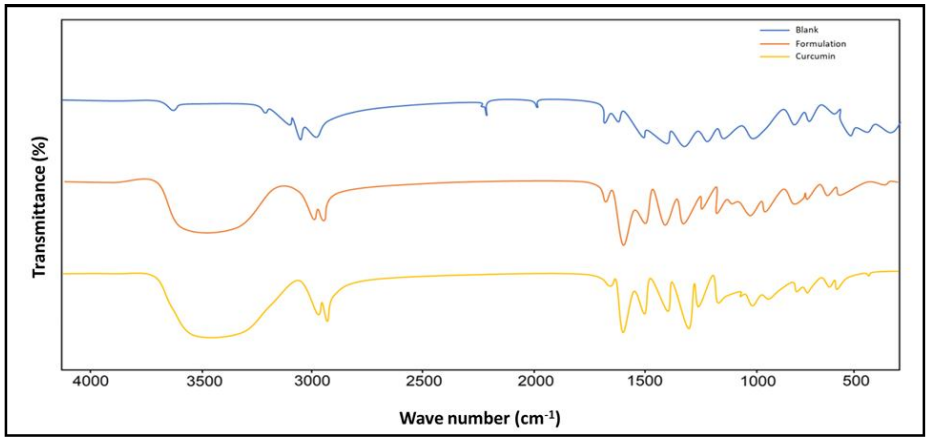


Figure 6: FTIR spectrum of blank formulation, curcumin and optimized formulation.

The FTIR results confirm the successful encapsulation of curcumin within the nanoemulsion. The reduction in intensity and slight shifts in the characteristic peaks of curcumin in the F5 formulation indicate that curcumin is not in its crystalline form but is likely dispersed or solubilized within the nanoemulsion matrix. The absence of phase separation in the spectra of the blank and F5 also suggests good compatibility between curcumin and the nanoemulsion components. Overall, the encapsulation enhances curcumin's solubility and stability, crucial for improved therapeutic efficacy.

3.5 *In vitro* DPPH antioxidant activity of optimized nanoemulsion

This study was performed to evaluate the scavenging effect of Avocado oil, a nanoemulsion formulation (F5), and ascorbic acid (standard reference). The results are represented as a percentage of inhibition at concentrations of 10, 20, and 30 µg/ml. At 10 µg/ml, the Avocado oil, nanoemulsion formulation, and ascorbic acid displayed inhibition percentages of 33.284 ± 5.348%, 51.893 ± 6.238% and 56.349 ± 6.233%, respectively, indicates that at this concentration, Avocado oil shows the lowest antioxidant activity compared to the formulation and ascorbic acid, which demonstrates that the nanoemulsion and ascorbic acid have a more potent radical scavenging effect at lower concentrations.

As the concentration increases to 20 µg/ml, the inhibition percentages for Avocado oil, nanoemulsion, and ascorbic acid increased to 49.380 ± 4.348%, 68.378 ± 9.378% and 72.783 ± 7.893%, respectively, suggests that the antioxidant activity of both the nanoemulsion and ascorbic acid improved significantly, with ascorbic acid remaining the most effective. However, the nanoemulsion formulation's activity approaches that of ascorbic acid, indicating that nanoemulsion enhances the antioxidant potential of Avocado oil.

At the highest concentration of 30 µg/ml, the inhibition percentages for Avocado oil, nanoemulsion, and ascorbic acid reached 58.348 ± 7.238, 73.238 ± 6.562% and 81.632 ± 6.993%, respectively, indicates a sustained increase in antioxidant activity across all samples, with ascorbic acid showing the highest activity, followed closely by the nanoemulsion. The nanoemulsion formulation demonstrated a significant improvement in antioxidant activity compared to pure Avocado oil at all concentrations tested.

Moreover, the results reveal that while avocado oil possesses inherent antioxidant activity, it is enhanced by the nanoemulsion formulation, bringing it closer to the potency of ascorbic acid. The formulation appears to stabilize and improve the radical scavenging efficiency of avocado oil, likely due to increased surface area and improved bioavailability in nanoemulsified form (Lavelli *et al*., 2021; Gaurav *et al*., 2022, 2023). Consequently, the nanoemulsion of Avocado oil presents a promising formulation for antioxidant applications, offering a potent alternative to ascorbic acid in various biomedical and nutraceutical applications. The results of the study have been depicted in Figure 7.

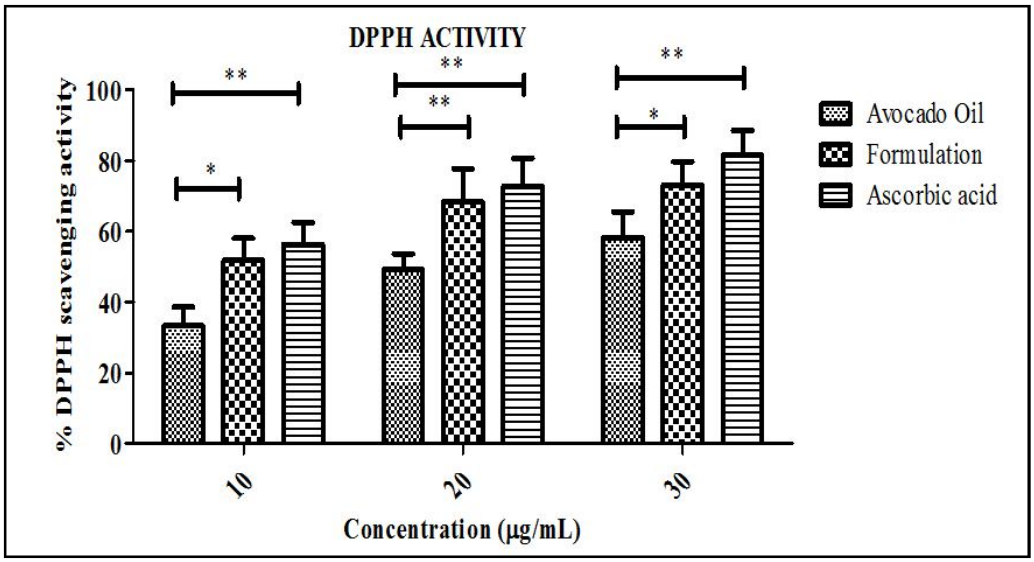


Figure 7: DPPH scavenging activity of Avocado oil, optimized formulation with ascorbic acid.

da Silva and his team conducted DPPH activity analysis demonstrated that oregano essential oil (OEO) nanoemulsions exhibit enhanced antioxidant efficacy compared to their non-nanoemulsified counterparts. The nanoemulsions, with droplet sizes of approximately 54.47 nm for OEO, showed over 45% DPPH radical inhibition, indicating significant improvement in antioxidant potential due to the nanoscale formulation ($p<0.05$). This enhanced bioactivity is attributed to the increased surface area and better dispersion of bioactive compounds, like carvacrol and thymol, in the nanoemulsion. These findings support nanoemulsions as a promising delivery system to enhance antioxidant activity for food and therapeutic applications (da Silva *et al*., 2023).

3.6 *In vivo* hepatoprotective studies

Hepatoprotective effect of developed and optimized nanoemulsion was determined against the hepatotoxicity inducted by CCl_4 administered through intraperitoneal route. The findings were presented through the analysis of liver biochemical markers, including AST, ALP, and ALT in blood serum, as well as oxidative markers like SOD, CAT, and MDA in the liver tissue homogenates.

3.6.1 Measurement of hepatic biomarkers

In this study, protective effects of various treatments, including nanoemulsion low dose (NELD), nanoemulsion high dose (NEHD), curcumin low dose (CLD), and curcumin high dose (CHD), was evaluated against CCl_4- induced hepatotoxicity. The hepatic biomarkers assessed are aspartate aminotransferase (AST, Figure A) alanine aminotransferase (ALT, Figure B) and alkaline phosphatase (ALP, Figure C), which are indicators of liver injury.

After administration of CCl_4, significant induction in the hepatic biomarkers was observed in the toxic group while administration of drug showed ameliorative effect on the elevated biomarkers. AST levels are elevated in the control group subjected to CCl_4, indicating liver damage. However, all treatment groups (NELD, NEHD, CLD, CHD) show a reduction in AST levels compared to the CCl_4 control, suggesting a protective effect. The high dose nanoemulsion (NEHD) and high dose curcumin (CHD) groups exhibit the most substantial reduction in AST, indicating that higher doses of nanoemulsion and curcumin may provide enhanced protection against hepatotoxicity. This implies that both nanoemulsion and curcumin can mitigate liver damage, with the nanoemulsion being potentially more effective at higher concentrations.

In ALT levels, CCl_4 treatment significantly increases ALT levels, signifying liver injury. However, treatment with NELD, NEHD, CLD, and CHD leads to a reduction in ALT levels, highlighting their hepatoprotective effects. Notably, NEHD and CHD demonstrate the lowest ALT levels among the groups, underscoring that higher doses of nanoemulsion and curcumin may offer superior protection. The marked decrease in ALT with NEHD compared to curcumin (CHD) further suggests that the nanoemulsion formulation could be more efficacious in reducing liver enzyme levels.

ALP, another indicator of liver health, show a substantial increase with CCl_4 administration, confirming hepatotoxicity. Treatment with NELD, NEHD, CLD, and CHD again leads to a decline in ALP levels compared to the CCl_4 group. High dose treatments (NEHD and CHD) are especially effective in reducing ALP, with NEHD showing the most significant reduction. This pattern reinforces the potential of nanoemulsion, particularly at higher doses, to provide hepato-protective benefits by lowering liver enzyme levels.

It has been reported that, the data suggest that both nanoemulsion and curcumin exhibit protective effects against CCl_4- induced liver toxicity. Nanoemulsion, particularly at a high dose, consistently shows greater efficacy in reducing AST, ALT, and ALP levels compared to curcumin, indicating a potent hepatoprotective capability. The enhanced bioavailability and targeted delivery provided by the nanoemulsion may account for its superior performance. These findings imply that nanoemulsion formulations, especially at higher doses, could be a promising therapeutic strategy for liver protection, potentially more effective than traditional curcumin treatment. This study highlights the advantage of nanoemulsion in enhancing the protective effects of curcumin against chemical-induced liver damage (Osawa, 2007; Park *et al*., 2010).

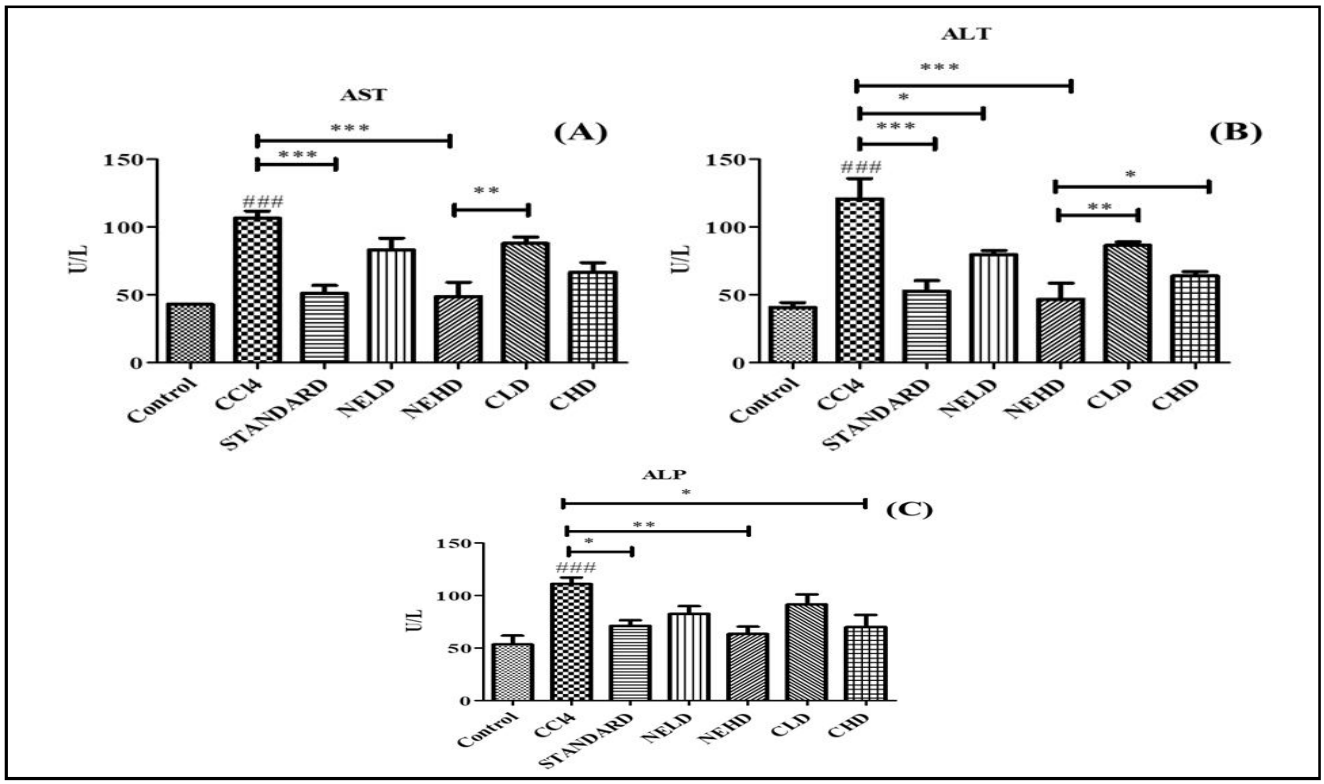


Figure 8: Assessment of hepatic biomarkers AST (Figure A), ALT (Figure B), ALP (Figure C) to evaluate protective effect of developed nanoemulsion (Low dose: NELD and High dose: NEHD), curcumin at low dose (CLD) and high dose (CHD) against CCl_4 induced hepatotoxicity. The results were expressed as Mean ± SD using ONE WAY ANOVA followed by Tuckey test to compare each pair of columns. The significance level was expressed at $p<0.05$ while summary represented as (#) mean comparison between control to toxicant and (*) represents toxic group to test group.

3.6.2 *In vivo* antioxidant activity

The effects of different treatments, including nanoemulsion low dose (NELD), nanoemulsion high dose (NEHD), curcumin low dose (CLD), and curcumin high dose (CHD), on oxidative biomarkers was evaluated in a model of CCl_4- induced oxidative stress. The biomarkers measured include indicators of oxidative damage and stress response, such as superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA).

In the case of superoxide dismutase (SOD), the CCl_4 control group shows a significant reduction in SOD activity when compared to the normal group, reflecting the oxidative stress caused by CCl_4 that weakens antioxidant defenses. However, treatment with NELD, NEHD, CLD, and CHD results in a considerable increase in SOD activity compared to the CCl_4 control, indicating that these treatments aid in the restoration of SOD levels. Notably, the NEHD and CHD treatments display the highest levels of SOD activity, with NEHD showing a slight advantage. This finding indicates that higher doses of both nanoemulsion and curcumin are more effective at enhancing SOD activity, but the high-dose nanoemulsion demonstrates superior protective effects, likely due to its improved bioavailability and cellular uptake.

In the case of catalase (CAT), a similar pattern is observed. The CCl_4 control group shows reduced CAT activity, which indicates diminished antioxidant protection and greater vulnerability to oxidative damage. Treatment with NELD, NEHD, CLD, and CHD leads to a recovery in CAT activity, with both high-dose treatments (NEHD and CHD) showing substantial improvements. However, NEHD results in the highest CAT activity levels among the treatment groups, highlighting the potential advantage of the nanoemulsion form in restoring catalase function. The enhancement of CAT activity by the nanoemulsion, particularly at high doses, emphasizes its ability to counteract oxidative stress more effectively than standard curcumin.

Malondialdehyde (MDA) levels serve as an indicator of lipid peroxidation and oxidative damage. The CCl_4 control group displays elevated MDA levels, reflecting significant lipid peroxidation and oxidative damage. All treatment groups exhibit reduced MDA levels compared to the CCl_4 control, indicating their protective effects against lipid peroxidation. The NEHD and CHD groups show the lowest MDA levels, with NEHD demonstrating a comparatively higher reduction than CHD. This finding suggests that the high-dose nanoemulsion is particularly effective in reducing oxidative damage, as evidenced by lower MDA levels, which signifies decreased lipid peroxidation.

Moreover, the results indicate that both nanoemulsion and curcumin treatments provide antioxidant protection against CCl_4- induced oxidative stress by enhancing SOD and CAT activities and reducing MDA levels. High-dose nanoemulsion (NEHD) consistently demonstrates greater efficacy in boosting antioxidant enzymes and reducing lipid peroxidation compared to curcumin alone. These findings underscore the potential advantages of nanoemulsion formulations in delivering curcumin, as the enhanced bioavailability likely contributes to improved protective effects. The study suggests that nanoemulsion-based curcumin formulations, particularly at higher doses, could be more effective for managing oxidative stress and protecting cells from oxidative damage than traditional curcumin treatments (Gaurav, 2022; Gaurav *et al*., 2022, 2023; Gautam, 2022; Salar *et al*., 2023).

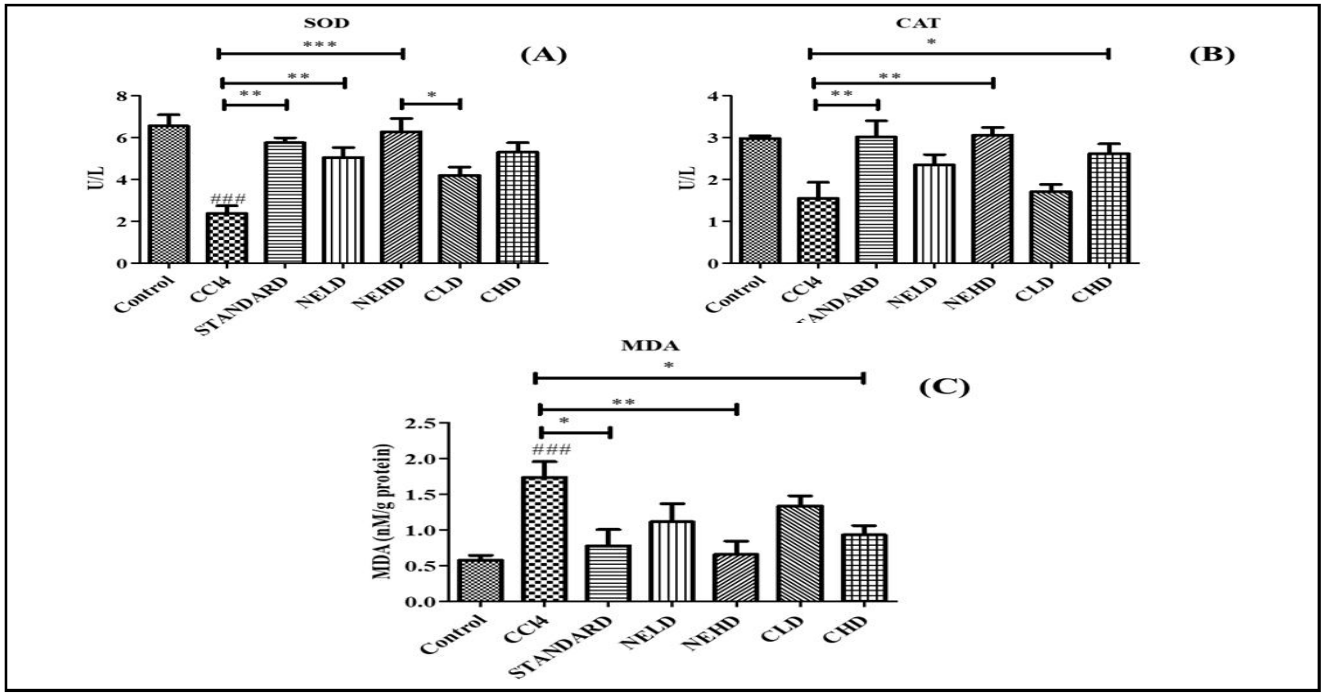


Figure 9: Assessment of oxidative biomarkers to evaluate protective effect of developed nanoemulsion (Low dose: NELD and High dose: NEHD), curcumin at low dose (CLD) and high dose (CHD) against CCl_4 induced oxidative stress. The results were expressed as Mean ± SD using ONE WAY ANOVA followed by Tuckey test to compare each pair of columns. The significance level was expressed at $p<0.05$ while summary represented as (#) mean comparison between control to toxicant and (*) represents toxic group to test group.

3.6.3 Histopathological assessment

Histopathological examination of liver sections from various treatment groups, including normal control, CCl_2 control, low-dose and high-dose nanoemulsion (NELD and NEHD), and low-dose and high-dose curcumin (CLD and CHD) was performed successfully. The outcome of study provide insight into the hepatic protective effects of the nanoemulsion and curcumin formulations against CCl_2 -induced liver damage.

The normal control group exhibits a typical liver histology with well-preserved hepatic architecture, uniform hepatocytes, and clearly visible central veins and sinusoids. In contrast, the CCl_2 control group shows significant histopathological damage characterized by cellular degeneration, vacuolization, and inflammatory cell infiltration, indicating severe hepatotoxicity due to CCl_2 exposure. The presence of large areas of cellular necrosis and disrupted liver structure highlights the extent of CCl_2 -induced oxidative damage.

The NELD and NEHD groups exhibit noticeable improvements in liver architecture compared to the CCl_2 control group. In the NELD-treated group, some recovery is evident with reduced inflammation and vacuolization, although mild degenerative changes remain. The NEHD group shows more pronounced protection, with relatively intact hepatocyte structure and reduced signs of necrosis and inflammation. This suggests that the high dose of nanoemulsion provides better hepatoprotection, likely due to enhanced bioavailability and antioxidant activity, which mitigate the oxidative damage caused by CCl_2.

Similarly, the CLD and CHD groups demonstrate protective effects, with the CHD group showing greater improvement than CLD. In the CHD group, liver tissue appears largely preserved with minimal inflammatory cell infiltration, suggesting that a higher dose of curcumin helps in restoring liver architecture more effectively. However, the NEHD-treated group shows comparatively better preservation of liver histology than CHD, indicating that the nanoemulsion formulation might be more effective at mitigating liver damage.

Moreover, the histopathological analysis demonstrates that both nanoemulsion and curcumin treatments reduce CCl_2 -induced liver injury, with high doses offering more significant protection. The enhanced efficacy observed in the NEHD group highlights the potential advantage of nanoemulsion-based curcumin for hepatic protection, possibly due to its improved absorption and potent antioxidant properties. These findings reinforce the potential therapeutic role of nanoemulsion formulations in managing liver injuries caused by oxidative stress. The histopathological examination has been represented in Figure 10.

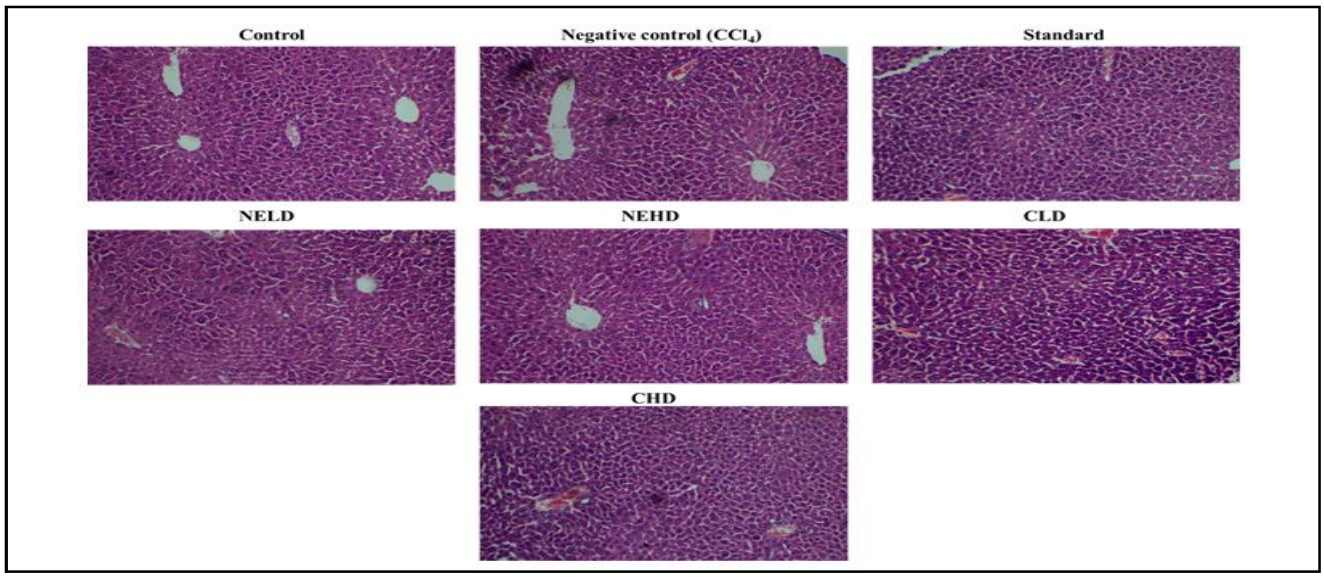


Figure 10: Histopathological sections of liver tissues from different treatment groups under 40x magnification stained with hematoxylin and eosin (H&E). Images show liver sections from (A) normal control, (B) CCl_4 control, (C) nanoemulsion low dose (NELD), (D) nanoemulsion high dose (NEHD), (E) curcumin low dose (CLD), and (F) curcumin high dose (CHD). NEHD shows minimal hepatic damage with preserved architecture, while the CCl_2 control exhibits pronounced hepatocyte degeneration, indicating severe damage. NELD and CHD display moderate improvements compared to CCl_2 control, but less protection than NEHD.

Agame and his team demonstrates the therapeutic potential of curcumin nanoemulsions in managing diet-induced hepatic steatosis, a prevalent metabolic disorder linked to excessive sweetened beverage intake. Curcumin nanoemulsions, stabilized by mono- and diacylglycerides with medium-chain fatty acids, were shown to improve liver health markers in a fructose-fed rat model. Notably, the nanoemulsions helped reduce body and liver weights, as well as serum cholesterol, LDL, AST/ALT, and HDL levels. Despite the lack of significant changes in serum glucose or triglycerides, histopathological analysis indicated a marked reduction in hepatic steatosis and inflammation. These findings highlight curcumin nanoemulsions' potential as a dietary intervention for fatty liver management (Agame-Lagunes *et al*., 2021).

In the case of superoxide dismutase (SOD), the CCl_4 control group shows a significant reduction in SOD activity compared to the normal

group, highlighting the disruption of antioxidant defense mechanisms due to oxidative stress induced by CCl_4. However, treatment with NELD, NEHD, CLD, and CHD results in a notable increase in SOD activity when compared to the CCl_4 control group, indicating that these treatments aid in restoring SOD levels. Additionally, Marwa and his team investigated the effectiveness of curcumin nanoemulsions as anti-inflammatory agents. Among the various formulations tested, the nanoemulsion containing 1% curcumin and 3% surfactant (F3) exhibited the best stability and performance. *In vivo* studies using carrageenan-induced paw edema in rats demonstrated significant antiinflammatory effects, with the 40 mg/kg curcumin nanoemulsion showing results comparable to the standard anti-inflammatory drug, ketorolac. These results suggest that curcumin nanoemulsions hold promise as a potential approach for the development of anti-inflammatory treatments (Marwa *et al*., 2023). This research highlights the promising potential of curcumin nanoemulsions as a cancer treatment. Although, curcumin faces challenges due to its low solubility in water and poor absorption, a nanoemulsion system enhanced with medium-chain fatty acids and lecithin successfully delivered the compound, achieving a particle size of 44 nm. The treatment led to a significant 91.81% reduction in tumor formation and an 89.95% decrease in tumor area in K14E6 transgenic mice, compared to untreated controls. Histological examination showed less inflammation and suppressed carcinoma progression, reinforcing the enhanced anticancer effectiveness of the nanoemulsion (Agame-Lagunes *et al*., 2020).

4. Discussion

This study uniquely leverages Avocado oil, rich in bioactive compounds and monounsaturated fats, as a lipid carrier. This is a novel approach to improving curcumin's bioavailability, which traditionally suffers from poor solubility and rapid metabolism. The developed nanoemulsion system demonstrated superior hepatoprotective effects, particularly at higher doses, compared to conventional curcumin formulations. This was evidenced by reduced liver biomarkers (AST, ALT, ALP) and improved histological preservation in the liver. The study showed that the optimized nanoemulsion formulation significantly enhanced the antioxidant activity of curcumin, as demonstrated by DPPH radical scavenging assays. This improvement underscores the potential for enhanced therapeutic outcomes. The use of FTIR spectroscopy in this study provided a detailed understanding of curcumin's interactions with nanoemulsion components, confirming stable encapsulation and compatibility, which contributes to the stability and efficacy of the formulation. The nanoemulsion formulation demonstrated excellent thermodynamic stability under various stress conditions, ensuring its applicability in diverse environments and potential for commercial scalability. By integrating curcumin with Avocado oil in a nanoemulsion, the study addresses dual challenges of poor bioavailability and therapeutic stability, offering a synergistic solution that enhances the therapeutic index of curcumin. The findings suggest that this formulation can provide effective hepatoprotection, potentially surpassing existing curcumin therapies. This highlights its potential for further development into clinical applications for managing liver-related diseases and oxidative stress.

5. Conclusion

The study evaluated the hepatoprotective potential of an avocado oil-based curcumin nanoemulsion system, with the best formulation, designated as F5, selected based on optimal pH, viscosity, encapsulation efficiency, and particle size. The selected nanoemulsion demonstrated promising antioxidant potential as evidenced by its DPPH activity, indicating its capacity to neutralize free radicals. For in vivo analysis, hepatoprotective effects were assessed using various groups: normal control, CCl_2 control (to induce hepatotoxicity), low-dose and high-dose nanoemulsion (NELD and NEHD), and low-dose and high-dose curcumin (CLD and CHD). Among these, the high-dose nanoemulsion (NEHD) exhibited the most significant protective effect against CCl_2 -induced hepatotoxicity and oxidative stress, outperforming both low-dose and standard curcumin treatments.

Histopathological and biochemical analyses confirmed that NEHD notably reduced markers of liver injury and maintained hepatic architecture, suggesting that the nanoemulsion's enhanced bioavailability and antioxidant properties provided superior hepatoprotection. Thus, the F5 curcumin-loaded nanoemulsion, particularly at a high dose, demonstrates great potential as an effective liver-protective agent.

The study highlights the advantages of nanoemulsion technology in enhancing the therapeutic efficacy of curcumin. Future research should explore the clinical application of this nanoemulsion formulation, including safety and efficacy in human trials. Additionally, investigations into its effects on other disease models, such as metabolic or inflammatory disorders, could further expand its therapeutic potential and applications.

Acknowledgements

The authors thanks to the School of Medical and Allied Sciences, KRMU, Gurgaon and for providing the facilities and expert support for completing the study.

Conflict of interest

The author declares no conflict of interest relevant to this article.

References

Afzal, M. F.; Khalid, W.; Armghan Khalid, M.; Zubair, M.; Akram, S. and Kauser, S. (2022). Recent industrials extraction of plants seeds oil used in the development of functional food products: A review. International Journal of Food properties, **25**(1):2530-2550.

Agame-Lagunes, B.; Alegria-Rivadeneyra, M.; Quintana-Castro, R.; Torres-Palacios, C.; Grube-Pagola, P. and Cano-Sarmiento, C. (2020). Curcumin nanoemulsions stabilized with modified phosphatidylcholine on skin carcinogenesis protocol. Current Drug Metabolism, **21**(3):226-234.

Agame-Lagunes, B.; Grube-Pagola, P.; García-Varela, R.; Alexander-Aguilera, A. and García, H. S. (2021). Effect of curcumin nanoemulsions stabilized with mag and dag-mcfas in a fructose-induced hepatic steatosis rat model. Pharmaceutics, **13**(4):509.

Ahmad, J.; Mir, S. R.; Kohli, K. and Amin, S. (2014). Quality by design approach for self nanoemulsifying system of paclitaxel. Science of Advanced Materials, **6**(8):1778-1791.

Ahmed, T. A.; Alotaibi, H. A.; Almehmady, A. M.; Safo, M. K. and El-Say, K. M. (2022). Influences of glimepiride self-nanoemulsifying drug delivery system loaded liquisolid tablets on the hypoglycemic activity and pancreatic histopathological changes in streptozotocin-induced hyperglycemic rats. Nanomaterials, **12**(22):3966.

Al Fatease, A.; Alqahtani, A.; Khan, B. A.; Mohamed, J. M. M. and Farhana, S. A. (2023). Preparation and characterization of a curcumin nano-emulsion gel for the effective treatment of mycoses. Scientific Reports, **13**(1):22730.

Algahtani, M. S.; Ahmad, M. Z.; Nourein, I. H.; Albarqi, H. A.; Alyami, H. S. and Alyami, M. H. (2021). Preparation and characterization of curcumin nanoemulgel utilizing ultrasonication technique for wound healing: *In vitro*, *ex vivo*, and *in vivo* evaluation. Reviews, **7**(4):213.

Ali, M.; Ain, S.; Kumar, B. and Ain, Q. (2022). Development and evaluation of eugenol-based gel formulation for analgesic and anti-inflammatory action. Ann. Phytomed., **11**(1):338-345.

Azeem, A., Rizwan, M., Ahmad, F. J., Iqbal, Z., Khar, R. K., Aqil, M. and Talegaonkar, S. (2009). Nanoemulsion components screening and selection: A technical note. Aaps Pharmscitech, **10**:69-76.

Ben-Shabat, S.; Yarmolinsky, L.; Porat, D. and Dahan, A. (2020). Antiviral effect of phytochemicals from medicinal plants: Applications and drug delivery strategies. Drug Delivery and Translational Research, **10**: 354-367.

Bobade, N. N.; Pande, S. D.; Tapar, K. K.; Ambalkar, P. V. and Malviya, S. P. (2016). Comprehensive review: Controlled release gastro-retentive muco-adhesive microspheres. Research Journal of Pharmacy and Technology, **9**(3):313-321.

Clower, L.; Fleshman, T.; Geldenhuys, W. J. and Santanam, N. (2022). Targeting oxidative stress involved in endometriosis and Its pain. Biomolecules, **12**(8):1055.

Council, I.; Harmonisation, F. O. R.; Technical, O. F.; For, R.; For, P. and Use, H. (2019). Biopharmaceutics classification system-based biowaivers M9. Internationalcouncil for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. pp:219-233.

Crucho, C. I. C. and Barros, M. T. (2017). Polymeric nanoparticles: A study on the preparation variables and characterization methods. Materials Science and Engineering C, **80**:771-784.

da Silva, B. D.; do Rosário, D. K. A.; Neto, L. T.; Lelis, C. A. and Conte-Junior, C. A. (2023). Antioxidant, antibacterial and antibiofilm activity of nanoemulsion-based natural compound delivery systems compared with non-nanoemulsified versions. Foods, **12**(9):1901.

Dhama, P. K.; Ain, S.; Kumar, B. and Ain, Q. (2022). Development and evaluation of topical ointment formulation containing gallic acid as an active pharmaceutical ingredient against bacterial infection and oxidative damage. Ann. Phytomed., **11**(1):439-449.

Dhanasekaran, S.; Rameshthangam, P.; Venkatesan, S.; Singh, S. K. and Vijayan, S. R. (2018). *In vitro* and *In silico* studies of chitin and chitosan based nanocarriers for curcumin and insulin delivery. Journal of Polymers and the Environment, **26**:4095-4113.

Duarte, J.; Sharma, A.; Sharifi, E.; Damiri, F.; Berrada, M. and Khan, M. A. (2023). Topical delivery of nanoemulsions for skin cancer treatment. Applied Materials Today, **35**:102001.

Gaurav (2022). GC-MS metabolomics and network pharmacology-based investigation of molecular mechanism of identified metabolites from *Tinospora cordifolia* (Willd.) miers for the treatment of kidney diseases. Pharmacognosy Magazine, **18**(79):230.

Gaurav; Khan, M. U.; Basist, P.; Zahiruddin, S.; Ibrahim, M. and Parveen, R. (2022). Nephroprotective potential of *Boerhaavia diffusa* and *Tinospora cordifolia* herbal combination against diclofenac induced nephrotoxicity. South African Journal of Botany, **151**:238-247.

Gaurav; Sharma, I.; Khan, M. U.; Zahiruddin, S.; Basist, P. and Ahmad, S. (2023). Multi-mechanistic and therapeutic exploration of nephroprotective effect of traditional ayurvedic polyherbal formulation using *in silico*, *In vitro* and *In vivo* approaches. Biomedicines, **11**(1):168.

Gaurav; Zahiruddin, S.; Parveen, B.; Ibrahim, M.; Sharma, I. and Sharma, S. (2020). TLC-MS bioautography-based identification of free-radical scavenging, α amylase, and α glucosidase inhibitor compounds of antidiabetic tablet BGR-34. ACS Omega, **5**(46):29688-29697.

Gautam, G. (2022). Network pharmacology-based validation of traditional therapeutic claim of momordica charantiain alleviating diabetic nephropathy Reviews, **1**:1-10.

Gharat, S.; Basudkar, V. and Momin, M. (2024). *In vitro* and *in vivo* evaluation of the developed curcumin-cyclosporine-loaded nanoemulgel for the management of rheumatoid arthritis. Immunological Investigations, **53**(3):490-522.

Gokkaya, E. O.; Yesilot, S.; Ozgocmen, M.; Aslankoc, R. and Aydin Acar, C. (2022). Protective effects of resveratrol and avocado oil against paracetamol-induced hepatotoxicity in rats. Drug and Chemical Toxicology, **45**(5), 2131-2139.

Hussain, A.; Samad, A.; Nazish, I. and Ahmed, F. J. (2014). Nanocarrier-based topical drug delivery for an antifungal drug. Drug Development and Industrial Pharmacy, **40**(4):527-541.

Jain, M.; Kapadia, R.; Jadeja, R. N.; Thounaojam, M. C.; Devkar, R. V. and Mishra, S. H. (2012). Amelioration of carbon tetrachloride induced hepatotoxicity in rats by standardized *Feronia limonia* Linn. leaf extracts. EXCLI Journal, **11**:250.

Jurenka, J. S. and Ascp, M. T. (2009). Curcuma anti-inflammatory properties. Alternative Medicine Review, **43**(4):589-616..

Khalil, H. M. A.; Mahmoud, D. B.; El-Shiekh, R. A.; Bakr, A. F.; Boseila, A. A. and Mehanna, S. (2022). Antidepressant and cardioprotective effects of self-nanoemulsifying self-nanosuspension loaded with hypericum perforatum on post-myocardial infarction depression in rats. AAPS Pharm. Sci. Tech., **23**(7):243.

Khan, M. U.; Basist, P.; Gaurav; Zahiruddin, S.; Penumallu, N. R. and Ahmad, S. (2024). Ameliorative effect of traditional polyherbal formulation on TNF-α, IL-1β and Caspase-3 expression in kidneys of wistar rats against sodium fluoride induced oxidative stress. Journal of Ethnopharmacology, **318**:116900.

Kumar, N.; Kumar, B.; Sisodia, V. K.; Rathour, A.; Roy, R. K. and Kumar, V. (2023). Determination of antibacterial and anti-inflammatory effect of developed ointment formulation containing rosmarinic acid, **12**:1-11.

Kumar, V.; Ain, S.; Kumar, B.; Ain, Q. and Gaurav (2020). Optimization and evaluation of topical gel containing solid lipid nanoparticles loaded with luliconazole and its anti-fungal activity. In: International Journal of Pharmaceutical Research, **12**(2):2901-2912.

Lavelli, V.; D'incecco, P. and Pellegrino, L. (2021). Vitamin d incorporation in foods: Formulation strategies, stability, and bioaccessibility as affected by the food matrix. Foods, **10**(9):1989.

Lin, T. K.; Zhong, L. and Santiago, J. L. (2018). Anti-inflammatory and skin barrier repair effects of topical application of some plant oils. International Journal of Molecular Sciences, **19**(1):70.

Marwa, A.; Iskandarsyah and Jufri, M. (2023). Nanoemulsion curcumin injection showed significant anti-inflammatory activities on carrageenan-induced paw edema in Sprague-Dawley rats Reveiws, **9**(4):310.

Osawa, T. (2007). Nephroprotective and hepatoprotective effects of curcuminoids. Advances in Experimental Medicine and Biology, pp:407-423.

P, N., S, S. and Rao, C. A. (2021). A complete review on mucoadhesive buccal tablets. Research Journal of Pharmaceutical Dosage Forms and Technology, pp:121-126.

Pandit, R. S.; Gaikwad, S. C.; Agarkar, G. A.; Gade, A. K. and Rai, M. (2015). Curcumin nanoparticles: Physicochemical fabrication and its *in vitro* efficacy against human pathogens. 3 Biotech, **5**:991-997.

Park, E.-J.; Jeon, C. H.; Ko, G.; Kim, J. and Sohn, D. H. (2010). Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. Journal of Pharmacy and Pharmacology, **52**(4):437-440.

S, J. P. and Evan Prince, S. (2018). Diclofenac-induced renal toxicity in female Wistar albino rats is protected by the pre-treatment of aqueous leaves extract of *Madhuca longifolia* through suppression of inflammation, oxidative stress and cytokine formation. Biomedicine and Pharmacotherapy, **98**:45-51.

Salar, S.; Gaurav and Sharma, P. (2023). Quality control and multi-targeted therapeutic approach of nyctanthes arbor-tristris for management of hepatic disease and associated complications. Pharmacognosy Magazine, **20**(1):57-71.

Schmidt, M., Bremer, E.; Hasenclever, D.; Victor, A.; Gehrmann, M. and Steiner, E. (2007). Role of the progesterone receptor for paclitaxel resistance in primary breast cancer. British Journal of Cancer, **96**(2):241-247.

Sheng, L.; Wei, Y.; Pi, C.; Cheng, J.; Su, Z. and Wang, Y. (2023). Preparation and evaluation of curcumin derivatives nanoemulsion based on turmeric extract and its antidepressant effect. International Journal of Nanomedicine, pp:7965-7983.

Velraj, M.; Patan, A.; Shanmugasundaram, P.; Jayakumari, S.; Vijayalakshmi, A. and Sundar, S. (2024). *In vitro* anticancer activity of root bark of *Mallotus philippensis* (Lam.) Muell. Arg. on cervical cancer cell line. Ann. Phytomed., **13**:522-528.

Zakir, F.; Ahmad, A.; Farooq, U.; Mirza, M. A.; Tripathi, A. and Singh, D. (2020). Design and development of a commercially viable in situ nanoemulgel for the treatment of postmenopausal osteoporosis. Nanomedicine, **15**(12):1167-1187.

Citation **Jyoti Kumari and Anoop Kumar (2024). Hepatoprotective potential of curcumin loaded Avocado oil-based nanoemulsion system. Ann. Phytomed., 13(2):544-558. http://dx.doi.org/10.54085/ap.2024.13.2.55.**