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Research Article
Enhancing the Anticancer Efficacy of Ciprofloxacin Towards Three Cancer Cell Lines via Avocado Oil Nanoemulsion Formulation

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Abstract

Background and Objective: Cancer is a serious disease that attacks different organs and causes a high rate of mortality every year, so we need developed more effective and safe anticancer agents. In the current study, a novel nanoemulsion formulation was designed to load ciprofloxacin into avocado oil nanoemulsion. **Materials and Methods:** The avocado oil was analyzed by GC-MS technique. The formed avocado oil nanoemulsion loaded ciprofloxacin was characterized by different criteria, zeta potential, PDI, entrapment efficiency, cumulative release and scanning electron microscopy. The GC-MS analysis resulted in the identification of 13 compounds. Oleic acid and linoleic acid were the major compounds in avocado oil. The avocado oil nanoemulsion loaded with ciprofloxacin was investigated as an anticancer drug against three cell lines; HepG2, MCF-7 and HCT116. **Results:** The avocado oil nanoemulsion loaded ciprofloxacin possesses high anticancer activity towards the three cell lines with IC_{50} of 4.55±2.23 μg mL^{-1} (for HCT116), 8.58±1.21 μg mL^{-1} for (HepG2) and 10.65±0.23 μg mL^{-1} for (MCF-7) showing remarkably improved results compared to ciprofloxacin alone and unloaded avocado oil nanoemulsion. Avocado oil nanoemulsion-loaded ciprofloxacin was found to arrest the cell cycles of HepG2, MCF-7 and HCT116 cell lines in the G2, G1 and G1 phases. There were 36.61±0.8 and 34.64±1.8% apoptosis increases of MCF-7 and HepG2 cells after avocado oil nanoemulsion-loaded ciprofloxacin treatment. **Conclusion:** The results favor applying avocado oil nanoemulsion-loaded ciprofloxacin as a powerful anticancer treatment.

Key words: Nanoemulsion, avocado oil, anticancer, GC-MS, apoptosis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Treatment of malignancies in the last decades is a progressive scientific field. Malignant tumors are considered life-threatening issues. Over the last decades, many studies have focused on designing new, effective, safe and applicable treatments to control cancer progression.

Nanotechnology was employed as a modern technique to develop new anticancer drugs. Nano drugs could be of metal origin, such as silver[1], gold[2], zinc[3] and iron nanoparticles[4]. Other nanomaterials employed for cancer treatments are nonmetallic and introduced as nanoformulations. The nanoformulations could be nanoemulsion, nanosuspension, nanopolymers[5], nanocomposites, niosomes[6], solid-liquid nanoparticles and nanofibers incorporating natural or synthetic polymers.

The application of loading a well-known drug from the market into new nanoformulations and applications towards new treatments is of great importance to skip the steps of toxicity and bioavailability studies[7].

Emulsions in the nanometer range are called nanoemulsions. Due to their advantageous properties, such as small size, high surface area per unit volume, enhanced dispersion of active hydrophobic components, improved absorption, nanoemulsion preparation and nanoemulsion stability[8], nanoparticles have many promising applications in the pharmaceutical industry because of their vast surface area, superficial charge, enhanced circulation half-life and precision targeting. Nanoemulsions have attracted the attention of cancer treatment researchers. Nanoemulsions spread rapidly in vascularized tissues because they migrate with cancer cells and, perhaps, employ their small size to their advantage. Nanoemulsions may significantly alter the way cancer is treated. These new formulations eliminate the solubility issue and preferentially destroy cancer cells.

The most potent fluoroquinolone, ciprofloxacin, is effective against various bacteria. It is notably effective against aerobic Gram-negative *Bacilli*, such as Enterobacteriaceae and Neisseria[9]. It is a fluoroquinolone from the second generation that has shown impressive versatility against a wide range of infections. With antibacterial solid action and a history of safe use, ciprofloxacin has excellent potential. Ciprofloxacin safety has been studied extensively and is supported by substantial evidence. Ciprofloxacin, first invented in 1983 by A.G. Bayer and then authorized by the Food and Drug Administration (US FDA) in 1987[10], represents a promising new option for anti-microbial treatment in pediatric populations. Brand names of ciprofloxacin are available in several countries.

One such broad-spectrum antibiotic is ciprofloxacin. Its antiproliferative and apoptotic properties in many cancer cell lines piqued the scientific community's attention. Several cell lines of cancer, osteosarcoma and leukemia were shown to be susceptible to its growth-inhibiting and apoptosis-inducing effects[11]. Ciprofloxacin was demonstrated to significantly impact the treatment of transitional cells in bladder cancer[12]. They also showed that ciprofloxacin inhibited the development of human prostate cancer cells by a combination of cell cycle arrest and death[13]. Topoisomerase II, topoisomerase IV and topoisomerase II are all inhibited by fluoroquinolones. Consequently, it has been shown that a number of fluoroquinolone antibiotics are highly cytotoxic to a range of mammalian cancer cell lines.

Natural products from plants, algae, animals and bacteria are essential to discovering new, safe treatments. The phytochemical constituents from the plant kingdom are responsible for the medicinal applications of natural plant constituents[14]. Concerning cancer, many studies demonstrated the use of total extracts and isolated compounds to stand against cancer progression[15,16].

Avocado fruit is the edible fruit of the evergreen tree of *Persea americana* (family Lauraceae). The fruit and the seeds of avocado were found to exert different pharmacological activities. It has anti-inflammatory[17], antioxidant[18], antidiabetic, hypolipidemic[19] and anticancer effects on other cell lines[20]. The avocado oil was extracted from the flesh of avocado fruit using different methods such as cold press, solvent, centrifugation, ultrasonic and supercritical extraction[21,22].

The current study was designed to augment activities of avocado oil and ciprofloxacin towards three cancer cell lines by incorporating the two in a nanoemulsion form as a novel drug delivery system to deliver ciprofloxacin to the target site of cancer and to evaluate the formed avocado oil-loaded nanoemulsion.

MATERIALS AND METHODS

Study area: This study was performed in the laboratories of Princess Nourah Bint Abdulrahman University, Saudi Arabia starting from October, 2022 till May, 2023.

Materials: Ciprofloxacin was obtained from (CAD Middle East Pharmaceutical Industries LLC, Riyadh, Saudi Arabia). Tween 80 and Span 20 were purchased from (Merck SA, Darmstadt, Germany) and avocado oil was obtained from (Saudi Green Industry, Riyadh, Saudi Arabia). The RPMI 1640, Gibco, USA,

was the source for the culture of human breast adenocarcinoma (MCF-7, liver carcinoma cells (HepG2) and colon adenocarcinoma (HCT116) cell lines. The FBS (10%) and 100 U mL^{-1} PS (penicillin/streptomycin) were supplied to that same media. The cells were incubated at 37°C in a humidified environment with 5% CO_2. Cell culture requirements were obtained from Gibco (USA). All other solvents and chemicals were of HPLC grade.

Gas chromatography

Sample preparation: A reaction between lipids and methanol that is catalysed by an alkali and carried out in the presence of 2 M potassium hydroxide and then injected in hexane results in the production of fatty acid methyl esters (FAME).

Gas chromatography: A flame ionization detector was installed onto an Agilent Technologies gas chromatograph (model 7890B). The separation was performed on a Zebron ZB-FAME column[22]. Briefly: After 3 min at 100°C, the temperature rose 2.5°C min^{-1} to 240°C for 10 min. One milliliter of fluid was injected. The injector and detector (FID) were kept at 250 and 285°C, respectively.

Ciprofloxacin solubility study: The solubility study of ciprofloxacin in avocado oil, Tween 80 and Span 20 was determined by adding an excess amount of ciprofloxacin to 1 mL of each of these substances (avocado oil, Tween 80 and Span 20) in separate vials. These vials were then placed in an isothermal shaker (Thermo Fisher Scientific, USA) at 24°C. After a 72 hrs incubation period, the samples were collected and centrifuged. The supernatant was carefully extracted and the concentration of ciprofloxacin was quantified using a UV spectrophotometer (Thermo Fisher Scientific, USA) at a wavelength of 278 nm.

Development of avocado oil nanoemulsion loaded with ciprofloxacin: A clear nanoemulsion was created by incorporating ciprofloxacin into avocado oil. This was achieved by gradually introducing a 3.5% Tween 80 and 1.5% Span 20 non-ionic surfactant into a warm 0.5% ciprofloxacin solution in distilled water. The mixture was stirred using a magnetic stirrer and produced a transparent solution. Next, 1.5% avocado oil was added to the surfactant-aqueous mixture while continuously stirring at 60°C. The continuous agitation facilitated the integration of avocado oil, resulting in a clear nanoemulsion. Finally, a series of heating and cooling cycles were conducted within an ultrasonicator for 90-120 min, alternating between 25 and 70°C. This resulted in a stable, transparent nanoemulsion enriched with ciprofloxacin[23].

Characterization of avocado oil nanoemulsion and ciprofloxacin-loaded avocado oil nanoemulsion

Particle size, zeta potential and polydispersity index (PDI) determination: The particle size, PDI and charge of the nanoemulsion were determined by a Malvern paranalytical (Zetasizer Nano ZS; Holtsville, New York, USA)[23].

Assessment of drug content

Entrapment efficiency (EE (%)): The drug content in the avocado oil nanoemulsion loaded with ciprofloxacin was determined with precision. To begin the assessment, a 1 mL aliquot of the formulation was dissolved in 10 mL of methanol. The mixture was then centrifuged at 5000 rpm for 30 min, resulting in a clear supernatant that was carefully collected for further analysis. The analysis was conducted using a spectrophotometric method, targeting the absorption peak at λ_{max} 228 nm. At this wavelength, the concentration of ciprofloxacin in each sample was closely monitored and accurately estimated. The cumulative ciprofloxacin release percentage was also determined. The entrapment efficiency (EE (%)) of ciprofloxacin in the avocado oil nanoemulsion was subsequently calculated, following the established procedure[6].

***In vitro* ciprofloxacin release:** With a diffusion area of 0.79 cm^2, a Franz diffusion cell was used for an *in vitro* drug release experiment. There was a dialysis membrane placed in between the donor and receptor compartments. As 1 mL formulation containing ciprofloxacin or a ciprofloxacin solution (concentrated at 3 mg mL^{-1}) was introduced into the donor compartment. Ciprofloxacin release was monitored on a regular basis for a duration of 15 to 300 min. Every 15 min, samples were taken following the method previously described with a little modification[5].

Biological study

Cell viability assay: Effects of ciprofloxacin monotherapy, ciprofloxacin-loaded nanoemulsion in avocado oil and avocado oil on cancer were evaluated using the sulforhodamine B (SRB) test[24]. To summarise, the following steps were taken prior to treating MCF-7, HepG2 and HCT116 cells with nanoemulsion formulations: Plating the cells at a density of 1×10^4 cells per well for 24 hrs. At 37°C, each formulation was incubated for 72 hrs with varying doses ranging from 0.01, 0.1, 1, 10 and 100 µg mL^{-1}. After 72 hrs, 150 µL of 10% trichloroacetic acid (TCA) was added to the culture medium and left at 4°C for 1 hr. It was washed five times with distilled water. Fifty microliters of SRB solution was used to stain the cells. Following that, 1% acetic acid was used

to remove any remaining discoloration. Each well was supplemented with 100 µL of a 10 mM Tris base solution at a pH of 10.5. Ultimately, a microplate reader set at 540 nm was used. The reader in question is a FLUOstar Omega from BMG Labtech in Ortenberg, Germany[24].

Cell cycle analysis: The D3 with IC_{50s} values were pre-calculated and delivered to cancer cells for 48 hrs. The cells were then trypsinized, washed twice in phosphate buffered saline (PBS), fixed in ice-cold 60% ethanol at 40°C and washed again in PBS. The cells were resuspended in 500 µL of propidium iodide (PI) with RNase staining buffer from cell signaling technology (CST) and incubated for 15 min. Finally, FACS analysis was performed using a Cytek® Northern Lights 2000 spectral flow cytometer (Cytek Biosciences), USA[25].

Apoptosis analysis: The HepG2, MCF-7 and HCT116 cells were treated with D3 for 48 hrs and then trypsinized and washed twice with PBS. Apoptosis assessment was done via the Annexin V-FITC/PI Apoptosis Detection Kit, cell signaling technology (CST), as stated by the manufacturer. The cells were applied to FACS analysis was performed using a Cytek® Northern Lights 2000 spectral flow cytometer (Cytek Biosciences) using SpectroFlo™ Software version 2.2.0.3 (Cytek Biosciences), USA[26].

Statistical analysis: All experiments were conducted in triplicate and the results were reported as Mean±SD. Student's t-test and One-way Analysis of Variance (ANOVA) were adopted to evaluate statistical significance using Graph pad Prism (version 7). The $p<0.05$ were regarded as statistically significant.

RESULTS

Fatty acids composition analysis of avocado oil by GC-MS: The analysis of lipodial composition was detected in the avocado oil sample used in the present formulation by gas chromatography coupled to mass spectrometry, a total number of 14 compounds were identified; the primary identified compounds were oleic acid with an area of 49.47%, followed by linoleic acid with area of 29.78%, of then palmitic acid with area of 10.38%, the GC-MS results were summarized in Table 1 and Fig. 1.

Table 1: GC-MS analysis results of avocado oil

Peak	RT (min)	Name	Area sum (%)
1	28.998	Palmitic acid	10.38
2	30.217	Palmitoleic acid	0.62
3	31.799	Margaric acid	0.01
4	34.54	Stearic acid	4.35
5	35.443	Oleic acid	49.47
6	37.177	Linoleic acid	29.78
7	39.296	Linolenic acid	4.52
8	39.713	Arachidic acid	0.30
9	40.462	cis-11-Eicosenoic acid	0.15
10	42.09	cis-11,14-Eicosadienoic acid	0.04
11	44.575	Behenic acid	0.21
12	46.89	Tricosanoic acid	0.02
13	49.155	Lignoceric acid	0.13

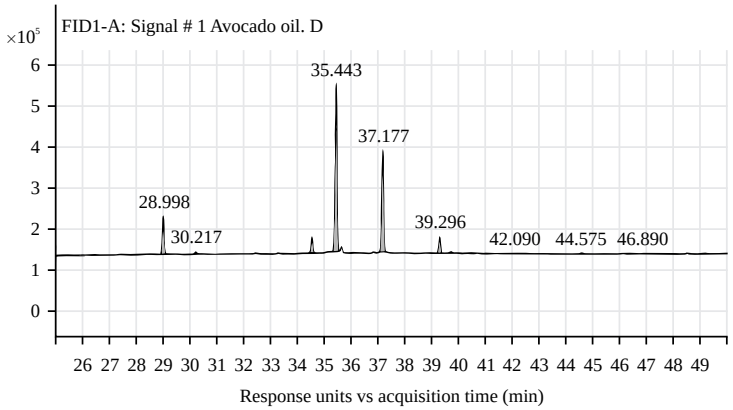

Fig. 1: GC-MS chromatogram of avocado oil

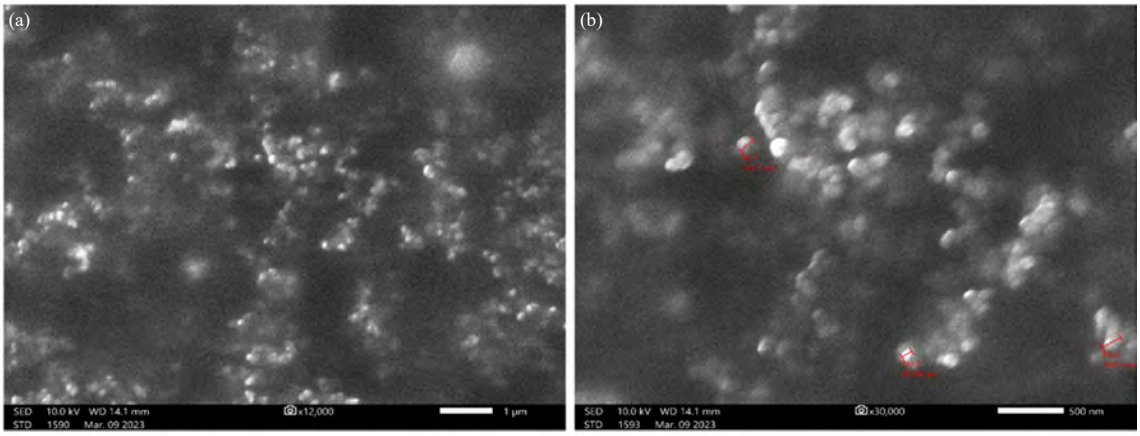

Fig. 2(a-b): SEM of the avocado oil nanoemulsion loaded ciprofloxacin at (a) X = 12000 and (b) x = 30000

Table 2: Zeta potential measurement of avocado oil nanoemulsion and avocado oil nanoemulsion loaded ciprofloxacin

Nanoemulsion formulations	Size (nm)	ZP (mV)	PDI
Avocado oil nanoemulsion (D1)	104.67±1.70	-21.33±1.25	0.12
Avocado oil nanoemulsion loaded with ciprofloxacin (D3)	191.00±3.27	-28.67±1.47	0.23

Data represent Mean±SD of three independent experiments and *p<0.05

Ciprofloxacin solubility study

Characterization of avocado oil nanoemulsion (D2) and avocado oil nanoemulsion loaded ciprofloxacin (D3)

Particle size, zeta potential and polydispersity index (PDI) determinations: Avocado oil nanoemulsion and avocado oil nanoemulsion loaded with ciprofloxacin (D3). A prepared nanoemulsion should be subjected to different characteristic techniques that measure the characteristics which prove a nanoemulsion formation, the particle size, particles charge and size distribution (which is measured by polydispersity index (PDI)). The formulated nanoemulsions sizes were 104.67±1.70 nm for avocado oil nanoemulsion and 191.00±3.27 nm for avocado oil nanoemulsion loaded ciprofloxacin, which was within the nanoparticulate range of 1000 nm, as summarized in Table 2. The addition of ciprofloxacin raised the nanoemulsion's size. The PDI values of the nanoemulsions were 0.12 and 0.23; both results are less than 0.8, indicating monodisperse samples. These PDI values assure stability and good dispersibility of the nanoemulsion droplets in avocado oil nanoemulsion and avocado oil nanoemulsion-loaded ciprofloxacin.

Zeta potential of nanoemulsion formulations: Surface charge, represented as zeta potential, is an additional important characteristic that affects the stability of the formulation and its biological activity. So, we found out how the nanoemulsions' surfaces were charged. The -21.33±1.25 and -28.67±1.47 mV zeta potential values for the avocado oil nanoemulsion loaded with ciprofloxacin and the avocado oil nanoemulsion without the antibiotic, respectively, were recorded (Table 2). These findings proved that the formulated D2 and D3 nanoemulsions were stable under dynamic conditions.

Entrapment efficiency (EE (%)) of ciprofloxacin loaded-avocado oil nanoemulsion: Entrapment efficiency (EE (%)) is a critical factor in the success of drug encapsulation. It serves as a measure of the formulation's ability to effectively incorporate a drug substance. The current study focused on the avocado oil nanoemulsion loaded with ciprofloxacin and examined its (EE (%)). Current study investigation revealed an impressive EE (%) of 96.33±0.91% for the formulation. This indicate an efficient drug-loading capacity exhibited by the nanoemulsion. The EE (%) value of 96.33% underscores the nanoemulsion's remarkable ability to efficiently accommodate ciprofloxacin.

Scanning Electron Microscope (SEM): Scanning electron microscopy was performed for the avocado oil nanoemulsion loaded with ciprofloxacin. The SEM analysis determines the accurate nanosize of the emulsion droplets, neglecting the surrounding environments, which were found in the range of 87-160 nm, the measured droplet size emphasizes a perfect nanoformulation (Fig. 2a-b).

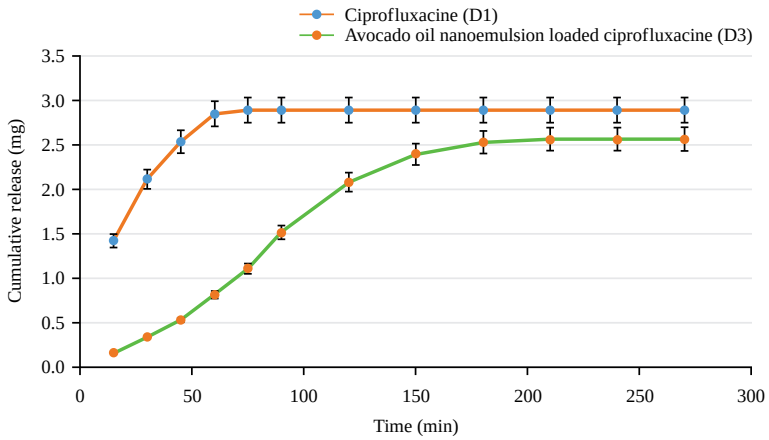

Fig. 3: Cumulative ciprofloxacin release of avocado oil nanoemulsion-loaded ciprofloxacin (D3) and non-formulated ciprofloxacin (D1) permeated through the membrane for 6 hrs

Each data point represents the Mean±SD (n = 3)

Table 3: IC_{50} of different formulations against different human solid tumor cells

	IC_{50} (μg mL^{-1})		
Formula	MCF-7	HepG2	HCT-116
D1	17.41±1.7	12.40±0.71	13.04±2.15
D2	25.82±2.72	32.44±5.95	31.54±1.40
D3	10.65±0.23	8.58±1.21	4.55±2.23

Data represent Mean±SD of three independent experiments

***In vitro* release study:** The *in vitro* release profile of ciprofloxacins from avocado oil nanoemulsion loaded ciprofloxacin (D3) was investigated using a Franz-type diffusion cell apparatus. As depicted in Fig. 2a-b, pure ciprofloxacin was released rapidly in the dissolution medium, with more than 96% of the drug released within the 1st hr. On the other hand, avocado oil nanoemulsion loaded with ciprofloxacin sustained drug release for up to 2.5 hrs. The percentage of drug release from avocado oil nanoemulsion-loaded ciprofloxacin was 84.1±1.9% after 150 min. These results are good evidence for improving the release of ciprofloxacin when incorporated into a nanoemulsion of avocado oil (Fig. 3).

Biological study

***In vitro* cytotoxicity of avocado oil nanoemulsion loaded ciprofloxacin:** The SRB assay was chosen to evaluate the cytotoxic potential of avocado oil nanoemulsion-loaded ciprofloxacin against MCF-7, HepG2 and HCT116 cells. The avocado oil nanoemulsion loaded with ciprofloxacin remarkably lowered the dose-dependent viability of all tested cell lines. The avocado oil nanoemulsion loaded with ciprofloxacin showed remarked anticancer activity towards the three cell lines under study with IC_{50} of 4.55±2.23, 8.58±1.21 and 10.65±0.23 μg mL^{-1} towards HCT116, HepG2 and MCF-7 cell lines (Table 3). Upon loading of ciprofloxacin on avocado oil nanoemulsion, a potentiation effect was noticed. The IC_{50} towards HCT116 was improved from 13.04±2.15 μg mL^{-1} for D1 and 31.54±1.40 μg mL^{-1} for D2 to 4.55±2.23 μg mL^{-1} for D3. The same action was observed for the HepG2 cell line where the IC_{50}s were 12.40±0.71 μg mL^{-1} for D1 and 32.44±5.95 μg mL^{-1} for D2 and improved to 8.58±1.21 μg mL^{-1} for D3. The same behavior was observed for the MCF-7 cell line where the IC_{50}s were 17.41±1.7 μg mL^{-1} for D1 and 25.82±2.72 μg mL^{-1} for D2 and improved to 10.65±0.23 μg mL^{-1} for D3. These results give evidence for the success of the formed avocado oil nanoemulsion loaded ciprofloxacin formulation for the potentiation of anticancer activity of ciprofloxacin and avocado oil towards the HCT116, HepG2 and MCF-7 cell lines in the same order and this is considered a high efficient formula that can be introduced in cancer therapy especially ciprofloxacin is a well-studied drug in the market that has well-known characteristics from the toxicity, bioavailability and pharmacokinetic points of view.

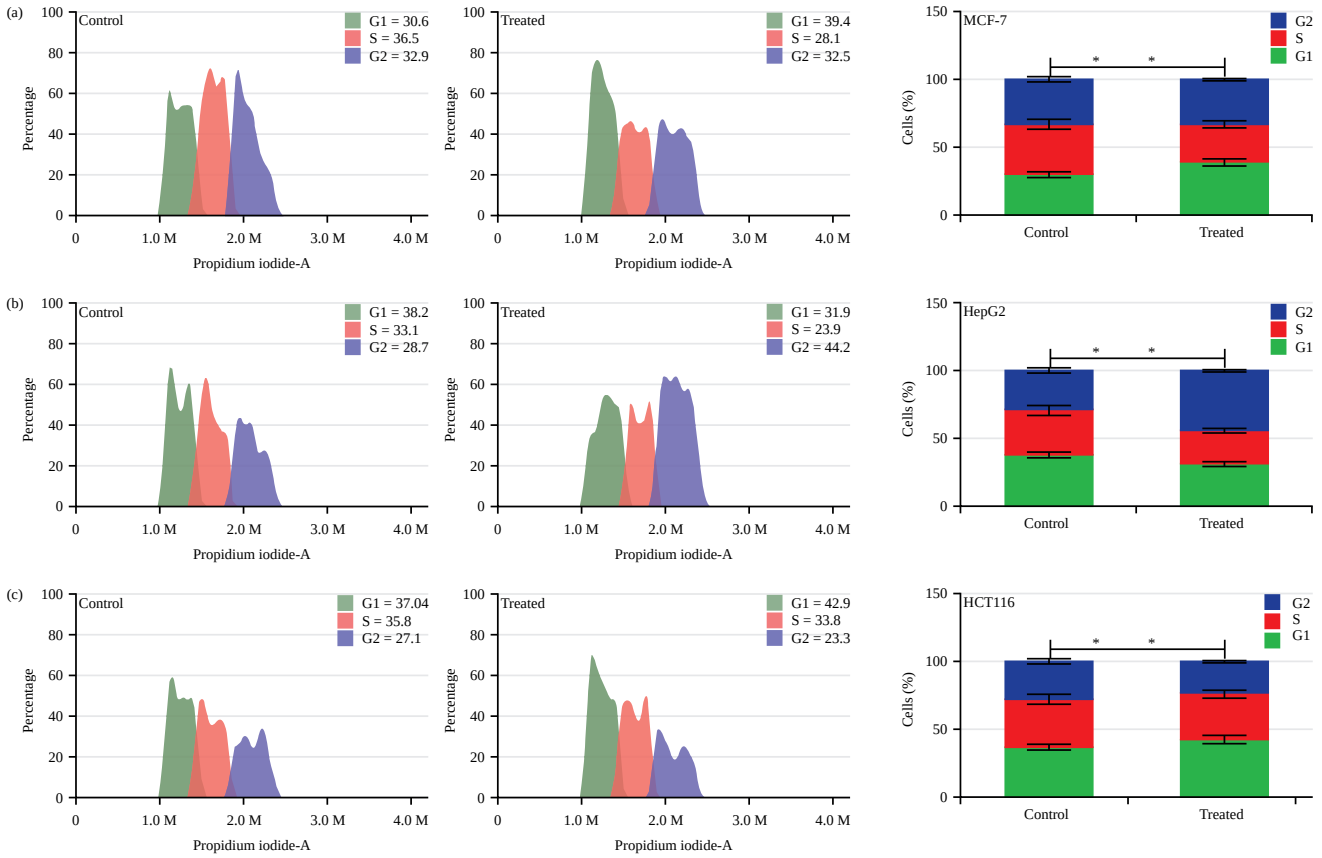

Fig. 4(a-c): Effect of avocado oil nanoemulsion loaded ciprofloxacin on cell cycle distributions of (a) MCF-7, (b) HepG2 and (c) HCT116 cells. Cell cycle distribution was determined using flow cytometry analysis

Data are presented as the Mean±SD, *p<0.05 and **p<0.01

Cell cycle analysis: Flow cytometry analysis was used to examine the impact of avocado oil nanoemulsion-loaded ciprofloxacin on different phases of cell cycles of each HCT116, HepG2 and MCF-7 cells. The avocado oil nanoemulsion loaded ciprofloxacin formulation significantly arrested the HCT116 in the G1 phase by a ratio of 42.9±2.1%. It arrested the HepG2 in the G2 phase by a ratio of 44.2±2.3%. The MCF-7 cancer cells were arrested in the G1 phase with a ratio of 39.4±1.4 % (Fig. 4).

Evaluation of cell apoptosis using annexin V-FITC: A flow cytometry analysis for the three cell lines (MCF-7, HepG2 and HCT116) treated with avocado oil nanoemulsion-loaded ciprofloxacin was performed after Annexin V-FITC staining. This analysis allowed the determination of the mechanism of the anticancer potential of the formulation in the form of necrosis or apoptosis. There was a 36.61±0.8 and 34.64±1.8% increase in apoptotic MCF-7 and HepG2 cells after avocado oil nanoemulsion-loaded ciprofloxacin treatment, respectively. The apoptotic effect in colon cancer cells (HCT116) was reduced to 28.38±0.8% after treatment with the combination formula, comparable to the apoptotic percent in MCF-7 and HepG2 cancer cells (Fig. 5).

DISCUSSION

The avocado oil nanoemulsion loaded with ciprofloxacin showed high stability as a nanoformula with zeta potential of -28.67±1.47 mV and PDI value of 191.00±3.27 nm. Ciprofloxacin, furthermore, the present study explored the apoptotic mechanisms induced by the avocado oil nanoemulsion loaded with ciprofloxacin in the tested cell lines. The results indicated a significant increase in the apoptosis rate, as demonstrated by the early and late apoptotic cell percentage. Specifically, MCF-7, HepG2 and HCT-116 cells showed apoptotic rates of 76.5±2.3, 78.3±3.2 and 74.46±1.9%, respectively, when exposed to the avocado oil nanoemulsion loaded with ciprofloxacin.

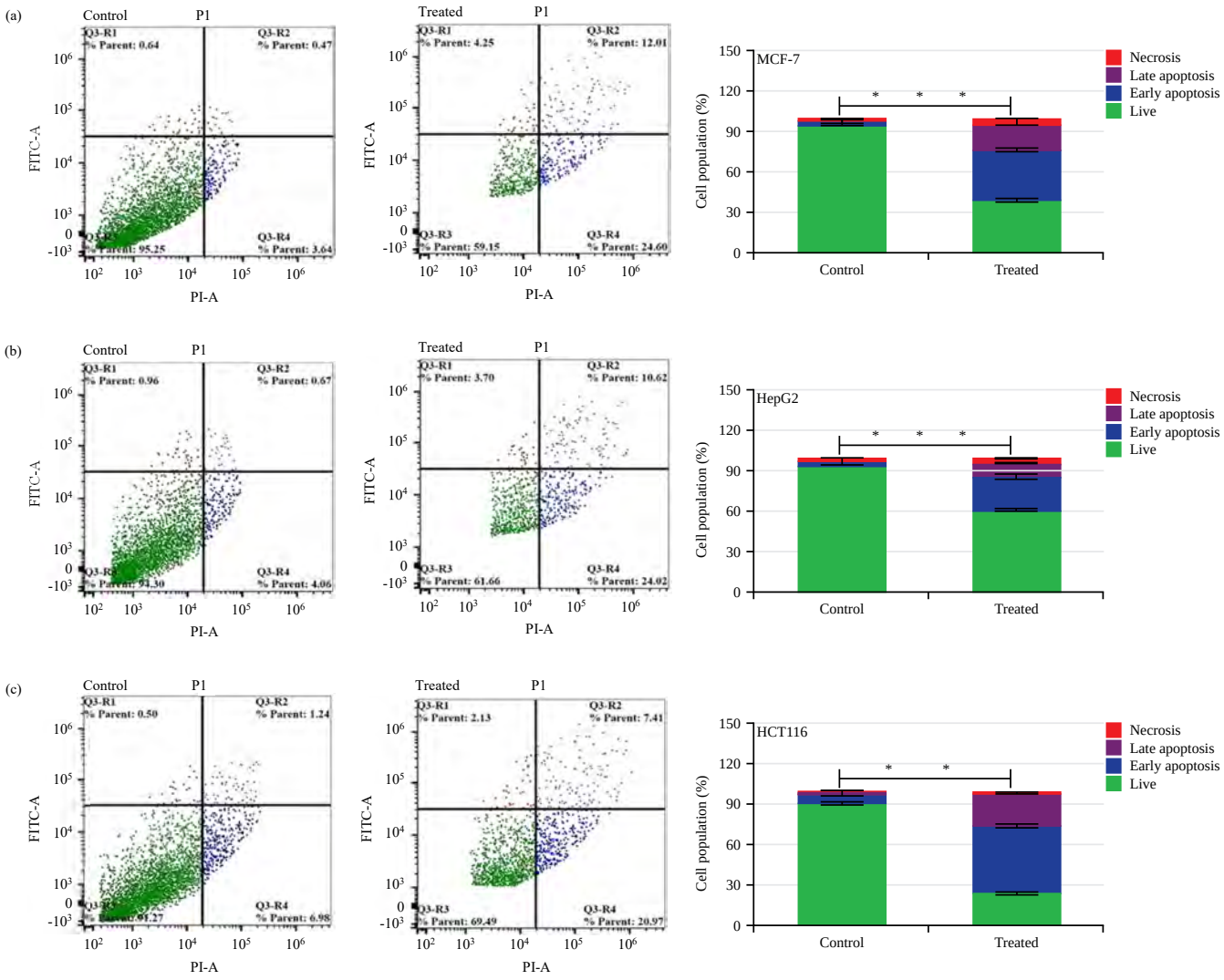

Fig. 5(a-c): Cellular apoptosis of (a) MCF-7, (b) HepG2 and (c) HCT116 treated with avocado oil nanoemulsion loaded ciprofloxacin for 48 hrs

Dot plots depict apoptotic cell distribution following Annexin V-FITC/PI staining, Column charts represent a quantitative evaluation of apoptotic cell percentage post-avocado oil nanoemulsion-loaded ciprofloxacin treatment. Data are presented as the Mean±SD

An FDA-approved antibiotic and a member of fluoroquinolones, has demonstrated effectiveness against various cancer cell lines[27,28]. Concurrently, avocado fruit, particularly its oil, has been investigated for its potential anticancer properties against several cell lines, including esophageal squamous carcinoma, colon adenocarcinoma and liver carcinoma[11,27].

The GC-MS analysis of the used avocado oil identified major fatty acids as palmitic acid, oleic acid and linoleic acid. These fatty acids were reported to have anticancer effects towards some cell lines. Palmitic acid suppresses gastric cancer development and metastasis by blocking the STAT3 signalling pathway[29]. Oleic acid was proved to possess anticancer effects towards liver cancer[30]. Oleic acid participates in the activation of many intracellular pathways involved in the growth of cancer cells[31]. Linoleic acid was proved to have anticancer potential against different cell lines in *in vivo* and *in vitro* investigations. Linoleic acid was effective against breast and melanoma cancer cells[32,33]. Based on the above results, avocado oil was a perfect candidate to be the oil phase to form a nanoemulsion loaded with ciprofloxacin.

Current study aimed to build upon these findings by combining avocado oil with ciprofloxacin in a nanoemulsion formula to investigate the synergistic anticancer effects of this combination. The resulting avocado oil nanoemulsion loaded with ciprofloxacin was carefully characterized, revealing a well-formed nanoemulsion with a zeta size of 191.00±3.27 nm. The polydispersity index (PDI) was measured at 0.23 and drug release studies indicated 84.1±1.9% release after 150 min.

The results obtained when tested the nano-formulated avocado oil nanoemulsion loaded with ciprofloxacin against three cancer cell lines: HCT116, HepG2 and MCF-7. Remarkably, the D3 formula exhibited significant anticancer potential, with IC_{50} values of 4.55±2.23, 8.58±1.21 and 10.65±0.23 μg mL^{-1} towards HCT116, HepG2 and MCF-7, respectively. When compared to ciprofloxacin alone (D1) and avocado oil nanoemulsion (D2), the avocado oil nanoemulsion loaded with ciprofloxacin (D3) demonstrated enhanced anticancer activity. These results are unique and are considered the first record to introduce ciprofloxacin in form of nanoemulsion. Other studies proved the anticancer effect of ciprofloxacin base[34] or when conjugated with oleic acid against PC-3 cell line[35]. Ciprofloxacin base was active against lung adenocarcinoma and ovarian cancer cell lines[36]. Chemical derivatization of ciprofloxacin led to active anticancer compounds when evaluated against T-24 and PC-3 cell lines[37,38]. The nanoemulsion formulations were found to enhance the anticancer efficiency of natural compounds[39] and it considered an excellent delivery carrier for antitumor medications[40].

CONCLUSION

Current study represents a notable advancement in drug discovery by harnessing the anticancer potential of ciprofloxacin through its incorporation into avocado oil nanoemulsion in combating liver, breast and colon cancers. The incorporation of ciprofloxacin into the avocado oil nanoemulsion significantly enhanced its anticancer potential beyond its antimicrobial properties. To bring this promising formulation to the forefront of anticancer medication, we recommend to perform additional research in the future such as pharmacokinetic and pharmacodynamic studies, be conducted to pave the way for its introduction into the drug market.

SIGNIFICANCE STATEMENT

This study was capable of introducing ciprofloxacin into a nanoemulsion formula to enhance the anticancer effect of ciprofloxacin towards three cancer cell lines, this achievement is produced for the first time and it is safe and efficient using a natural avocado oil. The formed nanoemulsion was able to stand against HepG2, MCF-7 and HCT116 cell lines and the mechanism of the anticancer was studied using the cell cycle analysis and apoptosis mechanism. The formed nanoemulsion was successful to perform its activities in very low dose compared to ciprofloxacin alone, this is considered a scientific achievement in the field of anticancer drug development.

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