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Fabrication, characterization, antimicrobial, toxicity and potential drug-delivery studies of PEGylated *Sesamum indicum* oil based nanoemulsion system

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Abstract

Background The actively mutating properties of disease-causing pathogens and GI intolerance associated with certain antibiotics among other challenges necessitated the adoption of colloidal system for drug delivery. Nanoemulsions (Ciprofloxacin (Cp) -loaded and non-drug loaded) were prepared by spontaneous emulsification method, characterized using Cryo-TEM, FTIR and Zetasizer. Antimicrobial activities were carried out using agar well diffusion method on *Klebsiella pneumoniae* and *Bacillus subtilis*. The in-vitro and dermal toxicological assessment were carried out using adult Wistar rats.

Results The Cryo-TEM micrographs showed spherical morphology while zetasizer results showed polydispersity index (PDI), mean droplet size and zeta potential (ZP) of 0.553, 124.3 ± 0.29 nm and − 15.3 mV respectively for non-drug loaded sesame oil-based emulsion (SOAB). While 0.295, 244.8 ± 0.33 nm and − 5.54 mV were recorded for Cp-loaded sesame oil-based emulsion (SOAB + Cp). The effective voltage charge of the emulsions was 147.4 V. FTIR results of Cp recorded O–H adsorption value of 3429 cm^{-1}, while SOAB and SOAB + Cp showed superimposition at 3427.76 cm^{-1} showing no drug-excipient interactions. No skin irritation was observed after 14 days of skin corrosion assessment. No significant difference ($p > 0.05$) in body weight gain of both test and control animals, the treatment did not cause any observable alterations in blood-chemistry parameters and hematological indices. Photomicrographs of liver and heart shows an uncompromised histological architecture.

Conclusion The finding of the study shows a skin friendly, nanosized, spherical negatively charged emulsion with no cardiotoxic, hematotoxic and hepatotoxic effects on Wistar rats, and as such appears promising as a safe vehicle for drug delivery.

Keywords Nanoemulsion, Histopathology, Bio-potency, Serum biochemsitry, Drug-delivery

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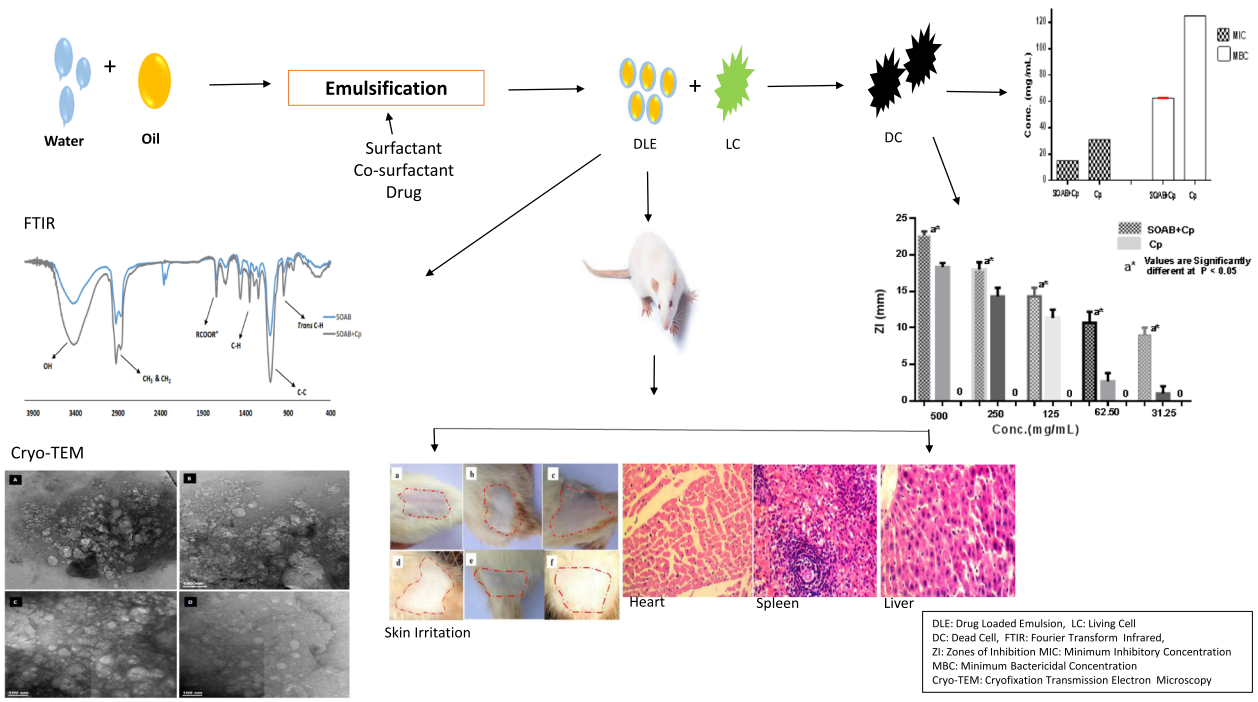
Graphical Abstract



1 Background

Patient compliance to drug most of the time is as a result of discomfort associated with its usage, which GI intolerance plays a major role. The use of emulsion to encapsulate bioactive materials for certain purposes, like taste masking and proper delivery has extensively been researched [1, 2] in which nanoemulsion has been flanked with numerous applications in nanomedicine and drug delivery purposes [3]. Colloids, especially the oil-in-water (O/W) type are charged particles [4] and as such, its affinity to cell membranes of disease causing pathogens due to the lipid nature of its surface is high as a result of similarity with the lipid-layered surface of the cell membranes of the cell. Its large surface area to volume ratio characteristics also accorded it an increase in permeation and penetration features thereby giving it the propensity to function as a vehicle for the delivery of drugs [5–9]. Sesame oil possess a nutritive, demulcent, and emollient properties which makes it to be of good pharmaceutical aid as solvent for intramuscular injections [10] and has a vast ethnobotanical applications [11]. It is non-toxic oil with a wide range of therapeutic properties. Its potential application in nanomedicine as the organic phase of nanoemulsion in drug delivery is due to the presence of unsaturated fatty acid: a reaction site which provides an anchorage for the hydrophobic ends of the surfactant molecule thus giving the oil a relevance in emulsification process for the production of nanoemulsion [12]. This is important as nanoemulsion plays a key role in modern pharmacy and medicine, providing series of means through which biomolecules could be effectively delivered into the body; oral, intravenous, nasal, ocular [6, 13] transdermal [14, 15] vaginal [16] among others [17]. *Klebsiella pneumoniae* is a gram negative saprophytic bacteria that lives on dead or decaying organic matter, it is also found in various environmental niches, like soil, water etc. as well as in GI tract. It is not only found in the GI tracts but at about 80%, a high carriage rates in nasopharynx of humans and skin, and other mammals [18, 19]. The increase in the colonization efficiency of *Klebsiella pneumoniae* is as a results of its resistance to antibiotics which enables the microorganism to persist and spread at a great rate, majorly in health care settings [20]. It is pathogenic in nature, especially in an immunocompromised individual and elderly people. This microorganisms has been known or labelled as one of the important causative agent of pneumonia which is one of the major community-acquired infections, other infections that could be traced to *Klebsiella*

pneumoniae is liver abscess, other metastatic and nosocomial infections [21, 22]. While *Bacillus* species are rod-shaped gram-positive (and sometimes turn gram negative) bacteria. They possess a wide range of physiological features owing to diversity in the gene which enables them to adapt and survive in every natural environment. Studies have shown that *Bacillus subtilis* which is one of the *Bacillus* species are found in the human GI tract [23] while some species are associated with bacteremia/septicemia (blood infections), endocarditis (heart infections), meningitis (spinal cord infections), and infections of wounds, the ears, eyes, respiratory tract, urinary tract, and GI tract [24–27] and as such, this microorganism should be considered gut commensals rather than solely soil microorganisms.

The use of drug (antibiotics) to lighten up microbial loads is as old as the existence of man on earth in which man uses leaves, tree backs, shoots and root to treat diseases. However, the greatest threat to the use of antibiotic is the ability of these disease-causing microorganism to evolve into a drug or multidrug resistant strains. Most of the pathogens overcome the epithelia and endothelia barrier to invade the body of their host [28] and replicates [29]. However, ciprofloxacin, a broad spectrum synthetic antibiotics belonging to the fluoroquinolones family has been known as a highly potent antibiotic. It exhibits catalytic inhibition mode of antimicrobial action by binding and inhibiting bacterial DNA gyrase which is responsible for DNA replication. It has been shown to be useful for the treatment of series of bacterial infections which includes upper and lower respiratory infections, soft tissue, skin infections as well as community acquired pneumonia [29–32]. Furthermore, the upward trend in the use of ciprofloxacin in community and hospitals over the years for the treatment of bacterial infections has resulted in the formation or the development of the drug-resistant species. It has been reported that occurrence of ciprofloxacin resistance in *K. pneumoniae* is now well established and, indeed, exceeds 5% in many centers in certain regions of the world [33].This therefore necessitates this study which is aimed at harnessing the excellent morphological features and encapsulating characteristics of nanoemulsion to enhance the therapeutic property of ciprofloxacin against drug resistant strains of disease-causing microorganism and also to develop a system that can help to evade GI tract discomfort associated with oral drug usage. More so, the sensitivity of the human body to xenobiotics (foreign substances) necessitates the need to also evaluate the toxicity or safety status of any materials to be ingested either for nutrition or therapeutic purpose. It is in this regard that the study examined the safety of *Sesamum indicum* oil based nanoemulsion system vis-à-vis its possible use as a vehicle for drug delivery, using adult male Wistar rats as experimental models. It is hoped that the outcome of the study will provide useful information on health implications as regards the use of SOAB for effective encapsulation of bioactive (drug) materials for effective transdermal, dermal and oral delivery without posing any threat to the delicate tissue of the body as well as the structure and texture of the skin.

2 Materials and methods

2.1 Materials

Polyethylene glycol (PEG 400) and Polyethylene (20) sorbitanmonooleate (T 80), Polyethylene (20) sorbitanmonolaurate (T 20) were purchased from Evergreen Chemical industry, Idumota Lagos, Nigeria. Sesame oil was extracted by Piteba mechanical extractor, Department of Chemistry, Federal University of Agriculture, Abeokuta Nigeria. The clinical isolates of the microorganisms were collected from Sacred Heart Hospital, Lantoro, Abeokuta, Nigeria.

2.2 Methods

2.2.1 Emulsification

The emulsification was carried out by weighing 0.075 mg (0.3%w/w) of the drug in a beaker containing 0.75 g (3% w/w) sesame oil and the mixture was homogenized until the drug was completely dissolved in the oil, then, 1.00 g (4% w/w) of T20, 4.00 g (16% w/w) of T80, and 1.00 g (4% w/w) of PEG 400 and 18.25 g (73% w/w) of distilled-deionized water were gently added. The mixture was homogenized using a magnetic stirrer (Faithful Huanghua SH-4C) at 800 rpm for 60 min [5, 34].

2.2.2 Droplet size and polydispersity index (PDI) measurement

The droplet size and PDI of O/W nanoemulsions were determined using zetasizer (Malvern Instruments, UK). Triplicate measurements were performed using He–Ne laser at a wavelength of 633 nm and at a scattering angle of 173° at 25 °C).

2.2.3 Drug-excipient interaction

The degree of crosslinking of the drug-excipient interaction was carried out using Fourier Transform Infrared (FTIR) spectroscopy (Schimadzu, Europe) on Cp, SOAB and SOAB + Cp by mixing the samples with KBr (Potassium Bromide, Spectroscopy grade), pelletize the mixture using hydraulic-press prior subjecting it to scanning.

2.2.4 Morphological characterization

The morphology of the emulsions were determined using Cryofixation-Transmission Electron Microscope

(Cryo-TEM). Cryo-grids were prepared using a Vitrobot (Thermo Fisher (FEI) Eindhoven, Netherlands). The sample (3 µL) was applied to a glow-discharged R2/2 copper Quantifoil grid (SPI, USA). The vitrified samples were stored in liquid nitrogen. While the cryo-samples were viewed using a FEI Tecnai F20 transmission electron microscope (Thermo Fisher (FEI), Eindhoven, Netherlands) with a field emission gun operating at 200 kV. Microscopy was done and images were recorded with a Gatan camera using the Digital Micrograph software suite (Gatan, UK).

2.2.5 Inoculums preparation

Each strain of bacteria was prepared overnight at 37 °C. The presence of turbidity in broth culture was adjusted equivalent to 0.5 McFarland standards to obtain standard suspension by adding sterile normal saline in Mueller-Hilton agar slants. The McFarland 0.5 standard provides turbidity comparable to bacterial suspension containing 1.5×10^8 CFU/mL and used at different dilutions in the proposed evaluation tests.

2.2.6 Determination of antibacterial activities

Antibacterial activities of the drug samples were evaluated by the well plate agar diffusion method using the earlier method with modifications [35]. The bacterial cultures were adjusted to equal 0.5 McFarland turbidity standards and inoculated on nutrient agar plate (diameters 9 cm) by flooding the plate with 1 mL of each of the standard test organism, swirled and excess inoculum was carefully decanted. A sterile cork borer was used to make wells (6 mm in diameter) on other agar plates. The different concentrations of 500, 250, 125, 62.50, 31.25 mg/mL of the drug, SOAB and SOAB + Cp were prepared by dilution method, and the antibacterial activity was determined by measuring the zone of inhibition around each well [36]. The MIC of both the drug and drug loaded emulsions were determined by the tube dilution technique, using a modified method [37]. Standardized suspensions which is a loopful of the test organism previously diluted to 0.5 McFarland turbidity standard were inoculated into a series of sterile tubes nutrient broth containing 250, 125, 62.50, 31.25, 15.07 and 7.53 mg/mL of Cp and SOAB + Cp respectively. Cross contamination was however prevented. The mixture was incubated for 24 h at 37 °C. Thereafter, the tubes were examined of visible sign of microbial growth. The smallest concentration that inhibits the growth was taken as the MIC [38]. The MBC is the lowest concentration of antibiotic agent that kills at least 99.9% of the organism. MBC of Cp and SOAB + Cp on the clinical isolate of *Bacillus subtilis* was carried out according to Doughari method [39]. 0.5 ml of the sample was removed from those tubes from MIC which did not show any visible sign of growth and inoculated on sterile Nutrient agar by streaking. The plates were then incubated at 37 °C for 24 h. The concentration at which no visible growth was seen was however recorded as the MBC of both the drug and the drug loaded emulsion [38].

2.2.7 Management of animals and experimental design

Ten adult male Wistar rats, weighing between 100 and 120 g were purchased from the animal breeding unit of the Department of Physiology and Pharmacology, College of Veterinary Medicine, FUNAAB. All procedures for maintenance and sacrifice (care and use) of animals were carried out according to the criteria outlined by the National Academy of Science published by the National Institute of Health [40], and approved by the Ethical Committee of the College of Sciences, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State. The animals were humanely handled, kept in plastic suspended cages in a well-ventilated and hygienic rat house under suitable conditions of temperature and humidity. They were provided with rat pellets (Arojo Feeds), and water ad libitum and subjected to natural photoperiod of 12 h light and 12 h dark cycle. They were randomly assigned to two groups, I and II containing five (5) animals each ($n = 5$).

The animals were acclimatized for two weeks before the commencement of the study. Group I animals served as control and group II animals were orally administered nanoemulsified *Sesamum indicum* oil (10 mL/kg bw) daily for a period of 21 days. After which they were sacrificed and blood samples and organs including the heart, liver and spleen were collected for analyses.

2.2.8 Biochemical assays

The serum activity of alanine amino transferase (ALT) and aspartate amino transferase (AST) was estimated spectrophotometrically (Asion, England) by Reitman and Frankel method [41] serum total protein and albumin levels were analysed using Randox kits (Randox Laboratories Limited, England) as described by Tietz [42]. The level of globulin was obtained by difference. Whole blood haemoglobin concentration was spectrophotmetrically (Asion, England) determined by Franco method [43] as described in cypress diagnostic Kit (Cypress Diagonistic, Belgium).

2.3 Histopathological analysis

The tissues collected for histopathology retrieved from each rat were preserved in buffer 10% formalin solution for histopathological processing and examination. The fixed tissues were labeled accordingly, dehydrated by passing it through ascending grades of ethyl alcohol (70%, 80%, 90% and 100%) solutions and cleared in xylene solutions baths to remove the alcohol, followed by infiltrating it in molten paraffin wax. Paraffin sections of the tissues were prepared and cut at 5 or 6 µm thick with a rotary microtome and stained with haematoxylin and eosin according to Avwioro's method the tissue sections were examined with light microscope [44].

2.4 Dermal irritation test

Skin irritation study involves the thorough evaluation of the skin in order to ascertain the safety of chemical substance, that is, its skin friendliness. The study was carried out in alignment with Organisation for Economic Co-operation and Development (OECD) skin irritation/corrosion test (methods) [45]. Total number of twelve (12) adult Wister rats were divided into three (3) groups comprising of four (4) rats each. A minimum of seven (7) days adaptation was allowed before the commencement of the study. Skin preparation was done twenty-four (24) hours before the test (dose application), hair on the back toward the tail of each rat were closely clipped exposing (approximately 6 cm area) of skin. A measured sample (0.5 mL) of SOAB and SOAB + Cp were evenly applied to a small area (approximately 6 cm square) of the closely clipped skin of each rat for four (4) hours exposure period, the area of administration were cleaned with wet cotton wool to remove any residual test substance. The test sites were scored for erythema, blanching, edema and ichthyosis at 1 h, 4 h, 24 h, 48 h, 72 h, 7 and 14 days post exposure with water (serving as control), SOAB and SOAB + Cp. Dermal responses were evaluated in accordance with OECD guideline.

2.5 Statistical analysis

The statistically significant difference between groups was analyzed using Independent sample *T*-test using Graph Pad Prism® (Version 6.04). The level of significance was set at $p < 0.05$. The results are presented as mean ± SD.

3 Results

3.1 Conductivity, zeta potential (ZP) and poly-dispersity index (PDI)

The droplet charge, size and shape of an emulsion are essential parameters as far as drug targeting and delivery is concerned, also in cosmetology. These values are essential in determining and forecasting the meta-stability of an emulsion system which is due to the reduction of the interfacialtension between the oil (dispersed phase) and the water (dispersant). The results of this study as presented in Fig. 1 shows an observable significant difference at $p < 0.05$ between the droplet size of SOAB (124.3 ± 0.29 nm) and SOAB + Cp, with a mean value of 244.8 ± 0.3 nm. With both emulsions recording an effective voltage charge value of 147.4 V [46].

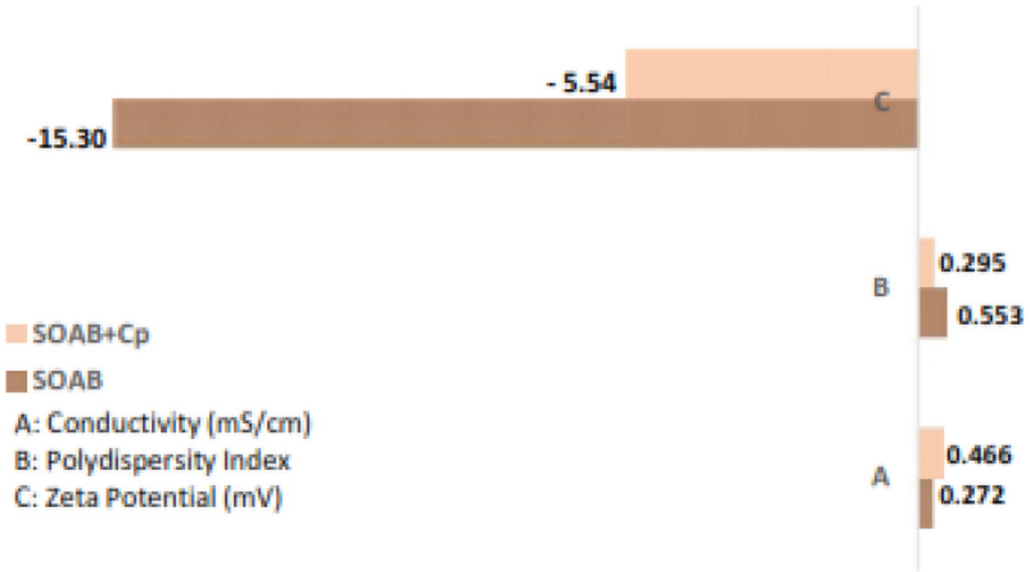


Fig. 1 Conductivity, polydispersity index (PDI), zeta potential of *Sesamum indicum* oil based nanoemulsion (SOAB) and Ciprofloxacin-loaded *Sesamum indicum* oil based nanoemulsion (SOAB + Cp)

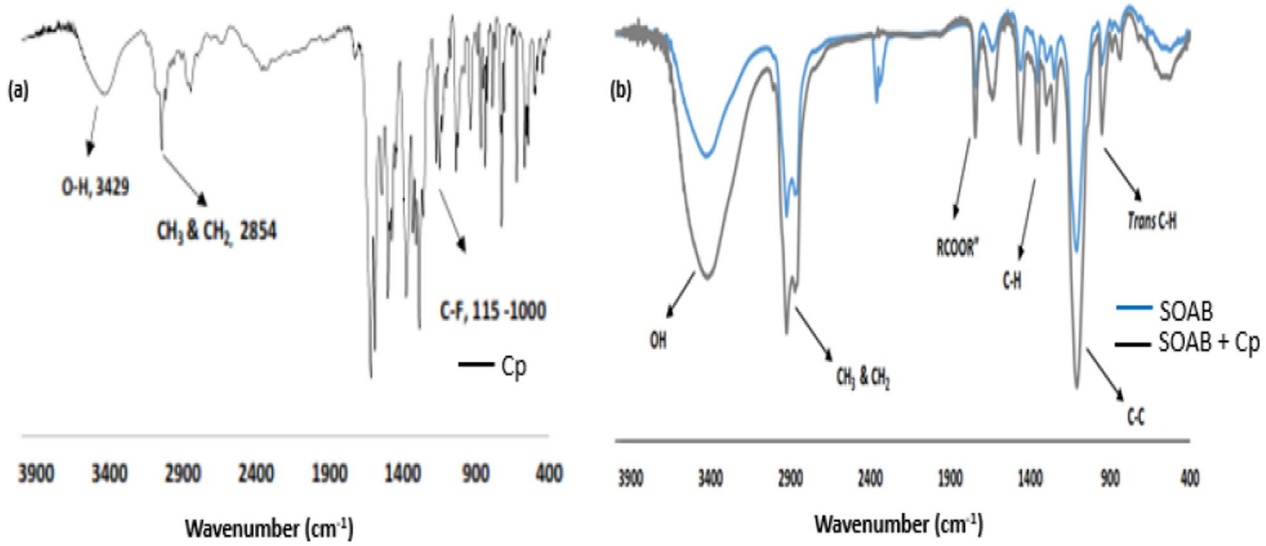


Fig. 2 FTIR Spectrum of **A**: Ciprofloxacin **B**: *Sesamum indicum* oil based nanoemulsion (SOAB) and Ciprofloxacin-loaded *Sesamum indicum* oil based nanoemulsion (SOAB + Cp)

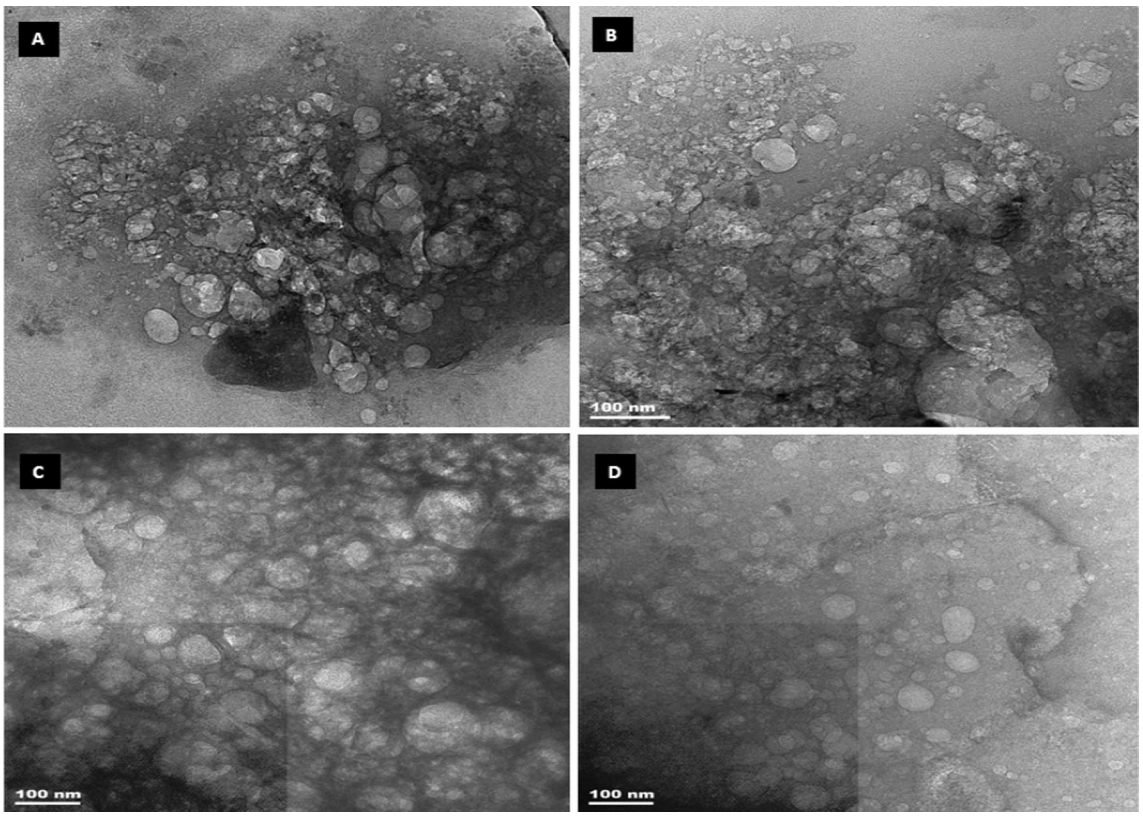


Fig. 3 The Cryo-TEM micrographs of **A**: *Sesamum indicum* oil based nanoemulsion (SOAB) **B**, **C** and **D**: Ciprofloxacin-loaded *Sesamum indicum* oil based nanoemulsion (SOAB + Cp)

3.2 Drug-emulsion interaction and morphological characterization

The morphological characterization of the emulsion and its surface chemistry showing drug-excipient interaction were examined by FTIR and Cryo-TEM as presented in Figs. 2a and b and 3a–d respectively. The FTIR analysis shows the functional groups in the drug (Cp), SOAB; the excipient, and the drug-excipient (SOAB + Cp) presented in Fig. 2. The spectrum of Cp (Fig. 2a) showed prominent absorption bands of O–H, CH_3 and/or CH_2 and C–F with a recorded absorption value range of 3570–3200, 2925–2845 and 1150–1100 cm^{-1} respectively. Upon comparing the spectra of SOAB and SOAB + Cp, a superimposition was noted with an absorption value of 3427.76 cm^{-1} suggesting a dimeric O–H group. While a prominent absorption band of symmetric S_p^2 and asymmetric S_p^3 was recorded at a wavenumber value of 2924.18 and 2070.17 cm^{-1} respectively which is characteristic absorption band of aliphatic hydrocarbon. The C–C absorption band which characterize *Trans* C–H absorption for out-of-plane bend was noted at a wave number value of 1109.11 cm^{-1} as presented in Fig. 2b. The spectrum of SOAB and SOAB + Cp showed a similar absorption spectra.

3.3 Antimicrobial study of Cp, SOAB and SOAB + Cp on *Klebsiella pneumonia* and *Bacillus subtilis*

The comparative bio-potency study of Cp, SOAB and SOAB + Cp on *Klebsiella pneumonia* and *Bacillus subtilis* presented in Fig. 4a–d shows that SOAB does not have any antimicrobial effect on both microorganisms' as shown in Fig. 5a in which ZI value of zero was recorded. Considering Cp and SOAB + Cp on *Klebsiella pneumoniae* at concentration range of 500–31.25 mg/mL of the formulation. It was observed that the mean ZI value decreases as the concentration of the formulation decreases. However, a significant difference was noted in the ZI value of Cp at $p < 0.05$ when

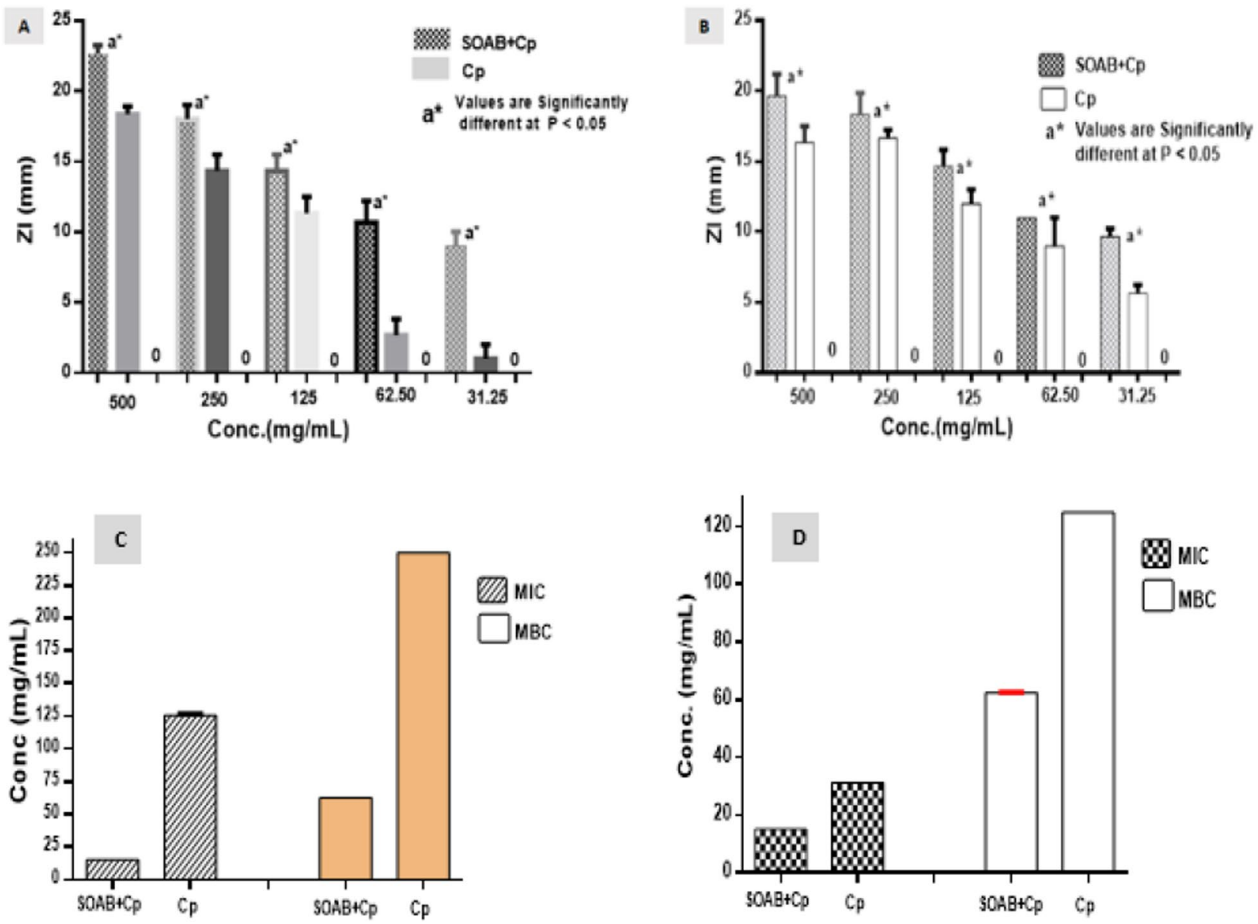


Fig. 4 The antimicrobial activities showing the Zones of Inhibition (ZI) of Ciprofloxacin-loaded *Sesamum indicum* oil based nanoemulsion (SOAB + Cp), Ciprofloxacin (Cp); and *Sesamum indicum* oil based nanoemulsion (SOAB) on **A**: *Klebsiella pneumonia* **B**: *Bacillus subtilis;* MIC and MBC of Cp and SOAB + Cp on **C**: *Klebsiella pneumonia* and **D**: *Bacillus subtilis*

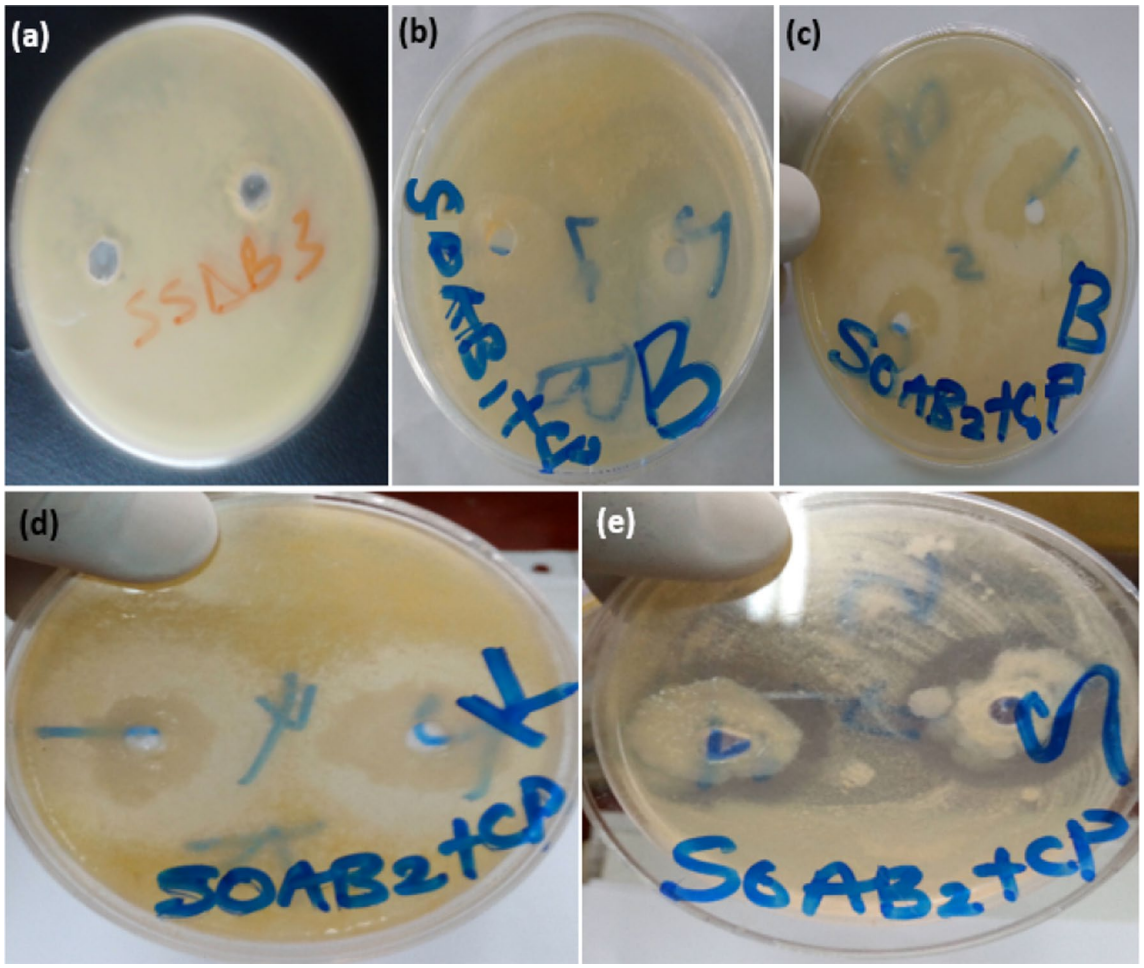


Fig. 5 Photographs showing the Zones of Inhibition (ZI) of **a** non-drug loaded *Sesamum indicum* oil based nanoemulsion (SOAB); **b**, **c**, **d** and **e** Ciprofloxacin-loaded *Sesamum indicum* oil based nanoemulsion (SOAB + Cp)

compared to SOAB + Cp in which the SOAB + Cp recorded a higher ZI value according to Fig. 4a. This suggests that SOAB + Cp has a better antimicrobial potency than Cp. Similarly, comparing the mean ZI values of both the drug and drug loaded emulsion on *Bacillus subtilis* at $p < 0.05$. The drug loaded emulsion system (SOAB + Cp) recorded a higher ZI values compared to Cp. This shows that SOAB + Cp has higher antimicrobial potential than Cp on *Bacillus subtilis* as shown in Fig. 4b. Furthermore, the comparative study of both MIC and MBC values of SOAB + Cp and Cp on both microbial strains shown in Fig. 4c and d recorded a low SOAB + Cp values which shows that the Cp has a low antimicrobial potential and less effective compared to SOAB + Cp on these bacteria strains.

Table 1 Effects of *Sesamum indicum* oil based nanoemulsion on average body weight of the experimental animals

	Conc. (ml/kg)	Initial weight	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16	Day 19	Day 21
C (kg)	Nil	133.25	139.50	150.00	156.75	163.75	169.00	175.75	175.00	177.25
SOAB (kg)	10	130.00	132.75	136.00	139.25	143.00	145.75	148.50	149.50	153.00
SOAB + Cp (kg)	10	132.00	134.00	139.75	142.00	147.25	151.00	154.50	153.00	157.00

Data are expressed as mean; n = 5

C Control, *SOAB Sesamum indicum* oil based nanoemulsion; *SOAB + Cp* Ciprofloxacin-loaded *Sesamum indicum* oil based nanoemulsion

Table 2 The serum biochemistry *Sesamum indicum* oil based nanoemulsion and ciprofloxacin-loaded formulations

Parameters	Control	SOAB	SOAB + Cp
Total protein (g/dl)	5.33 ± 0.67	5.05 ± 0.13	5.40 ± 0.16
Albumin (g/dl)	2.98 ± 0.10	3.13 ± 0.22	2.98 ± 0.22
Globulin (g/dl)	2.28 ± 0.75	1.78 ± 0.17	2.73 ± 0.39
T. Bilirubin (mg/dl)	0.65 ± 0.84	0.28 ± 0.15	0.80 ± 0.34
Urea (mg/dl)	7.75 ± 0.19	7.43 ± 0.29*	9.43 ± 0.56*
AST (U/L)	41.50 ± 6.46	47.00 ± 2.58*	57.75 ± 5.56*
ALT (U/L)	20.75 ± 3.30	27.25 ± 0.50	25.00 ± 3.65
ALP (U/L)	48.50 ± 19.64	69.50 ± 1.29*	66.50 ± 3.11*

Data are the mean of three replicates (n = 3): mean ± SD

*Shows significant difference at $p < 0.05$ for each experimental groups compared to the control group

Table 3 Effects of *Sesamum indicum* oil based nanoemulsion and ciprfloxacin-loaded system on haematological parameters on treated rats

Parameters	Control	SOAB	SOAB + Cp
PCV (%)	45.00 ± 2.94	52.00 ± 2.58*	49.50 ± 2.18*
Hb (g/dl)	14.10 ± 1.74	17.20 ± 0.25	15.40 ± 0.59
RBC × $10^{12/L}$	8.08 ± 0.92	8.65 ± 0.31	8.30 ± 0.26
WBC × $10^{9/L}$	5.65 ± 1.10	8.90 ± 0.26*	7.85 ± 2.02*
Neutrophils (%)	29.50 ± 9.26	39.50 ± 2.08*	34.50 ± 3.11
Lymphocytes (%)	67.50 ± 9.26	58.75 ± 1.71*	63.50 ± 2.08
Eosinophils (%)	0.50 ± 0.58	0.25 ± 0.50*	0.50 ± 0.58*
Monocytes (%)	1.50 ± 0.58	0.25 ± 0.50*	0.50 ± 0.58*
Basophils (%)	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
MCV (f/L)	56.06 ± 4.39	60.09 ± 0.85	59.62 ± 0.71
MCH (pg)	17.58 ± 2.32	19.90 ± 0.42	18.56 ± 0.22
MCHC(g/dl)	31.28 ± 2.42	33.12 ± 1.15	31.12 ± 0.50

Data are expressed as Mean ± SD

Hb hemoglobin; *HCT* hematocrit; *PCV* packed cell volume; *RBC* red blood cell; *MCV* mean corpuscular volume; *MCH* mean corpuscular hemoglobin; *MCHC* mean corpuscular hemoglobin concentration; *WBC* white blood cell; *SOAB Sesamum indicum* oil based nanoemulsion; *SOAB + Cp* Ciprofloxacin-loaded *Sesamum indicum* oil based nanoemulsion

*Values are statistically different at $p < 0.05$ for each experimental groups compared to the control group

3.4 Toxicity study

The effects of foreign material in the body system may results to unhealthy side effects which may include excessive weight gain/loss, damage to soft tissues particularly the liver, increased serum activity of enzyme biomarkers. Table 1 shows the effects of *sesamum indicum* oil based nanoemulsion on average body weight of the experimental animals. Tables 2 and 3 showed the effect of the *sesamum indicum* oil based nanoemulsion and ciprofloxacin-loaded formulations on serum biochemistry and haematological parameters on treated rats respectively. While Fig. 6 presented the visual effects of the emulsions both drug loaded and non-drug loaded on the heart, spleen, and liver of both the control and treated animals.

Blood-related indices such as total protein level, enzyme activity, hematological profile (leucocytes, erythrocytes, neutrophils, and hemoglobin and lymphocyte counts) are therefore used as primary pathological reflectors of the health status of humans and animals [47, 48]. Leucocytes, neutrophils, lymphocytes and monocytes are immunogenic parameters and integral components of the body`s defense mechanism against invasive pathogenic substances. However, if the invasive substance is persistently toxic, there is a subsequent decrease in the levels of these molecules in circulation via a process described as immunological suppression, leading to severe compromise in the normal physiological functions of the body system. This phenomenon increases the susceptibility of the affected organism to other toxins and pathogens with a possible attendant mortality. Furthermore, total plasma protein which is a sum of albumin and globulin plays vital roles in the effective functioning of the body's system. Hence, it is expedient that its plasma concentration exists within certain clinically defined range. Marked deviations from this physiological concentration range could trigger unhealthy metabolic state of the organism in question. The effect of the vehicle on blood related parameters are presented in Tables 2 and 3. Furthermore, SOAB and SOAB + Cp as observed in this study did not cause any significant and negative alterations in the hematological indices of the treated animals. Rather, administration of the nanomaterial boosted the leucocytes, hemoglobin and erythrocytes counts as well as the packed cell volume (PCV). An observable slight increase in hemoglobin and erythrocytes associated with of the treated animal when compared to the control in which a recorded mean value of 14.10 ± 1.74, 17.20 ± 0.25 and 15.40 ± 0.59% at $p < 0.05$ in control, SOAB and SOAB + Cp respectively. Furthermore, the increase in leucocytes and neutrophils count in rats after a period of 21 day administration of the test (SOAB and SOAB + Cp) samples when compared with the control animals recorded a mean higher value as presented in Table 2. The photomicrographs of the tissues presented in this study indicate that administration of SOAB to rats did not cause any notable structural aberration in the liver as substantiated by the photomicrograph of the treated animals (Fig. 6E) compared to the control counterparts (Fig. 6F).

3.5 Skin irritation study

Dermal compatibility of SOAB and SOAB + Cp as a potential vehicle for transdermal delivery of biomolecules was evaluated based on OECD guideline and the

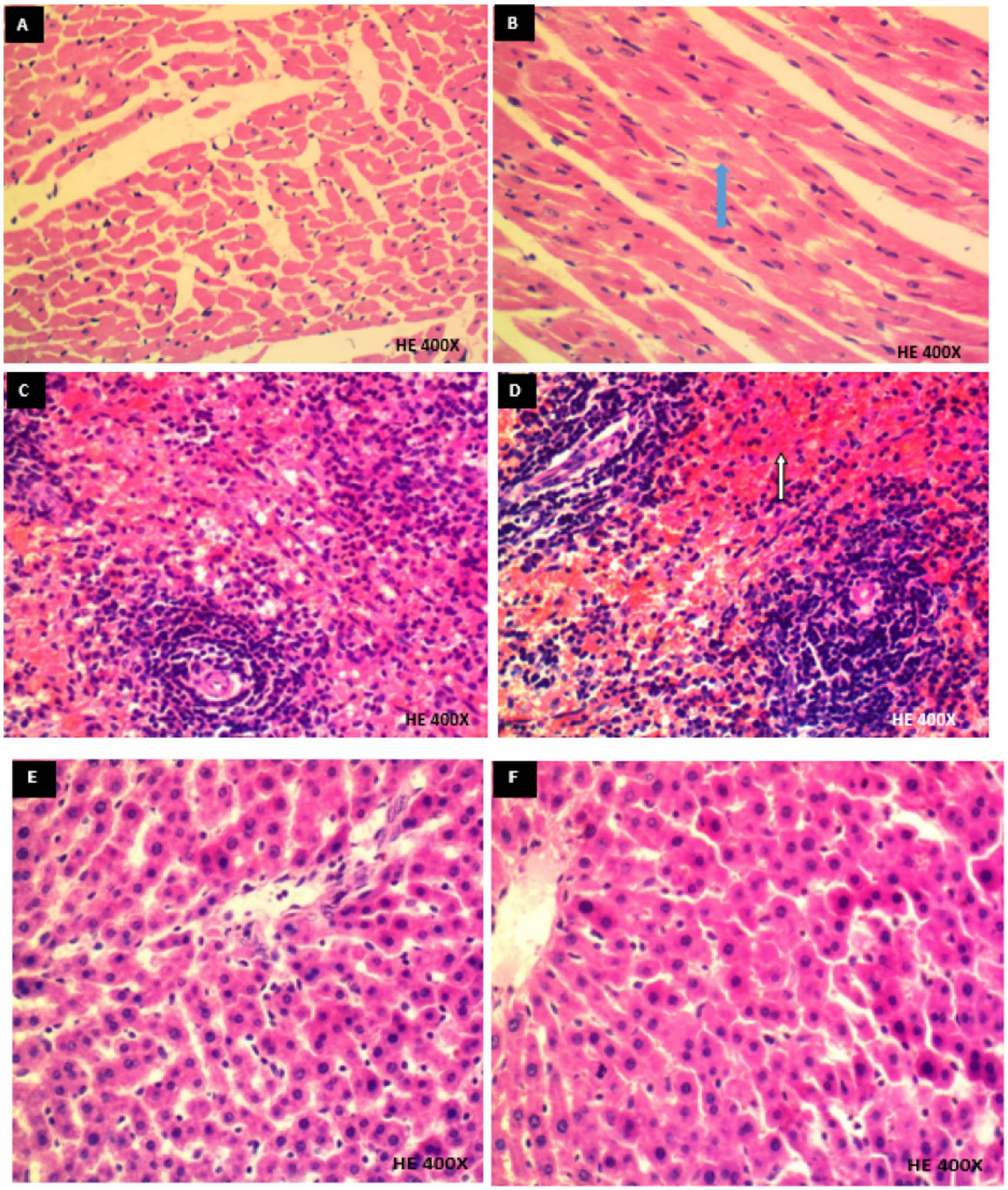


Fig. 6 Sections of the heart from control (**A**) showing normal myofibers and treated (**B**) rats shows an inflamed nucleated portion (blue arrow); Sections of the spleen from control (**C**) rats appearing normal and that from treated rats (**D**) showing severe congestion of the red pulp (white arrow); Sections of the Liver from control (**E**) and treated (**F**) rats of the liver appearing apparently normal

morphology of the portion treated with the emulsions vis-à-vis the control is presented in Fig. 7a–f after 14 days post administration. The result of the study shows that the integrity of the morphology of the skin was not jeopardized signifying no visible irritation (erythema, edema, ichthyosis, and blanching) or balding after application of SOAB and SOAB + Cp for 14 days on the rat skin.

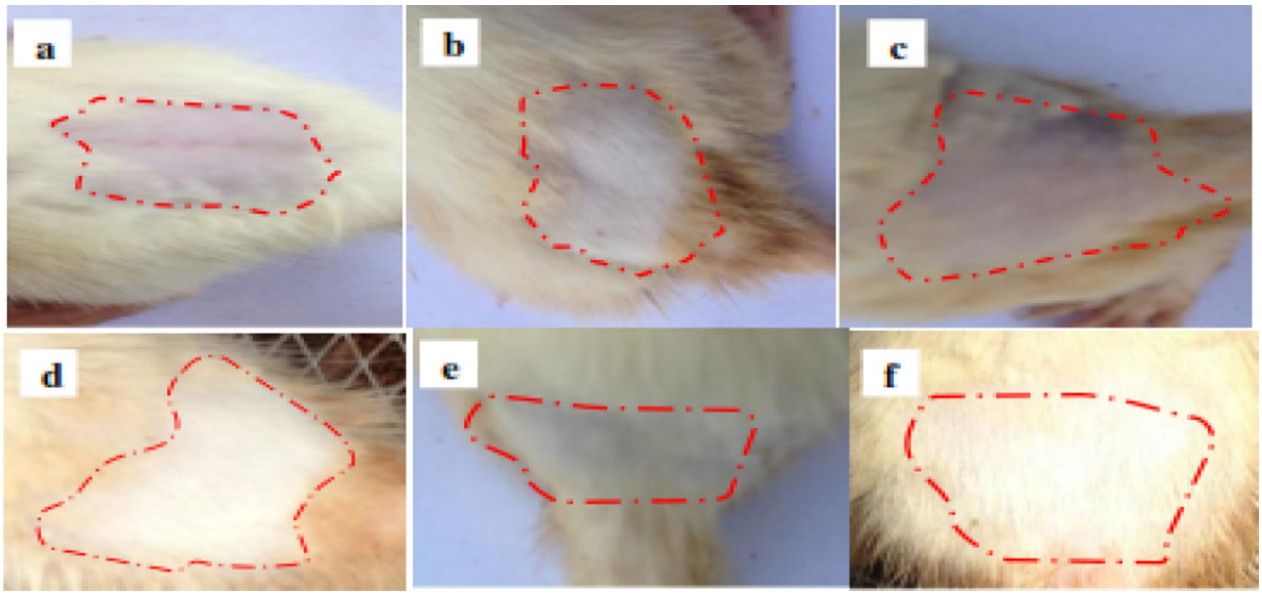


Fig. 7 **a**, **c** and **e**: are the pre-treatment at 0 day while **b**, **d** and **f** are the post-treatment of control, *Sesamum indicum* oil based nanoemulsion (SOAB) and Ciprofloxacin-loaded *Sesamum indicum* oil based nanoemulsion (SOAB + Cp) after 14 days of treatment

4 Discussion

The droplet charge, size, shape and PDI values of an emulsion are essential parameters that determines its probable application for drug targeting and delivery, and cosmetology. These parameters; size and charge, has been earlier reported to be responsible for electroporation of nanoemulsion vis-à-vis nanoparticle by providing the required electric field strength for the emulsion to effectively permeates through the keratinized lipid layered cell; transcorneocyte of the skin [49, 50]. Furthermore, PDI measures the dispersibility of an emulsion, its value ranges from 0 to 1. So, the recorded low PDI values in this study shows that the emulsions are roughly monodispersed. While ZP shows that the emulsions are negatively charged, which is as a result of PEGylation, due to PEG 400; a negatively charged stabilizing component of the emulsion system [51]. These properties fosters rapid permeation rate, enhance cellular uptake, opsonization and phagocytosis of nanomaterials in the living cells as well as massive removal by fixed macrophage [34, 49].

The FTIR analysis shows that there is no drug-excipient interaction (cross-linking) between the encapsulated Cp and SOAB. This suggests an effective encapsulation of the drug by the emulsion system with SOAB serving as a suitable reservoir. More so, studies have shown that nanomaterial with spherical morphology have been reported to show higher metastability, with an improved rate of cell permeation. Cryo-TEM micrographs of the emulsions; SOAB and SOAB+Cp in this study showed a spherical morphology. This corroborates the recorded value of the emulsion droplet size, PDI and effective voltage charge (EFC) as reported by Ajay [49] that an emulsion with EFS value more than 100 V tend to be more stable.

It has been reported that charged surface enhances rapid cellular uptake [52–54] also lipid-layered surface facilitates attraction between the lipid-layered cell membranes of bacterium cell and the colloidal system [7, 50]. This thereby suggests that the improved therapeutic characteristic of the encapsulated Cp in SOAB system; SOAB + Cp could be due to surface modification- charge, shape and size- of the encapsulated drug in the emulsion phase. Therefore, this enhanced the penetration and permeation rate of the drug-carrying-emulsions thereby facilitating opsonization and phagocytosis thus leading to the cell lysis of the bacterium cell treated with SOAB + Cp [1, 6]. This is in accordance to the study of Bamisaye et al. [34] on *E. coli* and Al-Adham et al. [21].

Furthermore, some of the unhealthy side effects of drug or drug-vehicles include excessive weight gain/loss, which may result to a damage to soft tissues particularly the liver [48, 51]. Also, this could results to an increased serum activity of enzyme markers such as ALT, AST and ALP. High levels of bilirubin and urea, as well as marked decrease in white blood cells and hemoglobin content due to lyses of red blood cells are a pointer to certain instability or disturbance to the proper functionality of the body internal system [55, 56]. In the present study, treatment of animals with SOAB and SOAB + Cp did not cause any weight loss or excessive weight gain in the test animals. The control rat, though showed relatively higher

body weight gain, this however did not differ significantly from the recorded weight values in the test animals, which is SOAB and SOAB + Cp. This observation suggest that the use of nanoemulsified *Sesamum indicum* oil and its drug loaded counterpart as a vehicle does not have the propensity to interfere with nutrient absorption and utilization, factors which are evidently critical to body weight gain or loss.

The hematological parameters shows that SOAB and SOAB + Cp did not cause any significant and negative alterations in the hematological indices of the treated animals. Rather, administration of the nanomaterial boosted the leucocytes, hemoglobin and erythrocytes counts as well as the packed cell volume (PCV). An observable slight increase in hemoglobin and erythrocytes associated with the treated animal when compared to the control could be ascribed to some level of erythropoietic effects to the nanoemulsions as well as the ability to promote oxygen-carrying capacity of blood in organisms. The trend observed in the haemoglobin parameter is also noted in the RBC value, in which SOAB recorded the highest mean RBC value compared to the control and SOAB + Cp. Since iron is a vital precursor in the formation of hemoglobin which in turn is required for red blood cells production and function (oxygen binding or distribution). It is suggested that SOAB is rich in iron. This postulation is consistent with previous opinions that the blood system of animals may improve in their iron content and oxygen-carrying capacity following treatment with certain substances [56–58].

The observable significant difference in the leucocytes and neutrophils at $p < 0.05$ between the control and treated animals could probably be due to a triggered immune response following a prolonged exposure of the animals to the foreign substance. Treatment of rats with SOAB and SOAB + Cp in the present study did not cause any obvious alterations in the physiological levels of total protein, globulin and albumin in the test animals compared to those of their control counterparts. This observation further attests to the apparent safety of SOAB as a potential vehicle for drug delivery and targeting. Moreover, the liver is arguably the most susceptible among the vital organs in the body [4]. During hepatic metabolism, toxic substances and free radicals capable of causing structural damage to the liver are readily generated, resulting to the leakage of ALT, AST and ALP from the cytosol of the hepatocytes into circulation [4, 59]. These enzymes, particularly ALT and AST are clinical markers of liver function. Furthermore, ALP is a clinical index which is not peculiar to the liver but also associated with several other organs, including the spleen [60]. The increase in the levels of ALP noted in the test animals is however a cause for concern. This may be connected to the severe congestion noted in the pulp of the spleen of these animals. It is attributable to mild obstruction of the biliary duct in effort of the body to get rid of the metabolized SOAB [61]. The congestion in the biliary duct possibly occurred due to prolonged administration of the nanoemulsion. However, it should not be a major concern; since in any case the use of vehicles in drug delivery and targeting does not require a prolonged administration as employed in this study.

Erythema is the redness of the skin caused by hyperemia of superficial capillaries [62]. Edema means, swelling caused by fluid in the body tissue, Ichthyosis is the scaling of the skin while blanching is whitening of the skin in response to chemical substances. The transdermal toxicity result of this study was evaluated based on the OECD guideline in which no reaction = 0, very slight reaction (barely perceptible) = 1, well defined reaction = 2, Moderate to severe reaction = 3 and severe reaction = 4 [45]. Draize et al. [63] reported that biomolecules or compounds producing scores less than 2 are considered negative, which is, no skin irritation which was modified by Ramanunny et al. [64]. The result of this study is in accordance with the work of Harwansh [65]. However, it is suggested that the formulations are not toxic to the skin of Wistar rat based on OECD scale [45].

Generally, the major limitation associated with colloidal systems as earlier reported is, instability [66, 67]. This could be due to Ostwald ripening, creaming, agglomeration, sedimentation and breaking owing to irregular morphology (size and shape) of the organic phase of the colloidal system which could either be monodispersed or polydispersed in nature. This phenomenon is shown by PDI value at a range of 0–1 as earlier stated. However, the PDI value, size, shape and charge of the emulsion produced in this study shows a stable nanoemulsion system with the potential to serve as a vehicle for biomolecule encapsulation and effective delivery.

5 Conclusion

The study shows that sesame oil based nanoemulsion and its drug loaded counterpart recorded a low PDI values which suggests high stability, a negatively charged surface with spherical morphology in nanometer range which have the propensity to enhance the penetration and permeation rate of the encapsulates through the cell membranes of a bacterium cell due to rapid cellular uptake. The ciprofloxacin loaded emulsion shows better antimicrobial potentials compared to the drug alone with an increase in ZI, and low MIC and MBC on *Klebsiella pneumoniae* and *Bacillus subtilis* respectively. Furthermore, the investigated dosage (10 mL/kg bw) of SOAB and SOAB + Cp has no cardiotoxic, hematotoxic and hepatotoxic effects on Wistar rats. However, its

induced-congestion in the spleen pulp remains a slight cause for concern if it were to be used in chronic administration. While the skin irritation evaluations shows that the emulsions, both the drug and non-drug loaded are skin friendly without any irritating effect on the skin of Wistar rats. The findings of this work shows that the formulations has an encapsulating potential thus making it suitable candidate for oral, transdermal and particularly dermal delivery of drugs. Albeit, further study is necessary in order to determine the pharmacodynamics and transdermo-pharmaceutic potential of the encapsulated drug in the living system.

Abbreviations

Cp	Ciprofloxacin
Cryo-TEM	Cryo-fixation transmission electron microscope
FTIR	Fourier transform infrared spectrophotometer
SOAB	Nanoemulsified *Sesamun indicum*
SOAB + Cp	Ciprofloxacin loaded nanoemulsified *Sesamum indicum*
GI	Gastrointestinal
PEG	Polyethylene glycol
PDI	Poly dispersity index
CFU	Colony forming unit
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
ZP	Zeta potential
HCT	Hematocrit
PCV	Packed cell volume
RBC	Red blood cell
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
WBC	White blood cell
RBC	Red blood cell

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Declarations

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Consent for publication
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Competing interests
The authors declare no conflict of interest.

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References

1. Sonneville-Aubrun O, Simonnet JT, L'Alloret F (2004) Nanoemulsions: a new vehicle for skincare products. J Adv Colloid Interfacia Sci 108(109):145–149. https://doi.org/10.1016/j.cis.2003.10.026
2. Mei Z, Chen H, Weng T, Yang V (2003) Solid lipid nanoparticle and microemulsion for topical delivery of Triptolide. Eur J Biopharm 56:189–196. https://doi.org/10.1016/S0939-6411(03)00067-5
3. Bouchemal K, Briançon S, Perrier E, Fessi H, Bonnet I, Zydowicz N (2004) Synthesis and characterization of polyurethane and poly (etherurethane) nanocapsules using a new technique of interfacial poly condensation combined to spontaneous emulsification. Int J Pharm 269(1):89–100. https://doi.org/10.1016/j.ijpharm.2003.09.025
4. Kim AY, Berg JC (2000) Fractal heteroaggregation of oppositely charged colloids. J Colloid Interface Sci 229(2):607–614. https://doi.org/10.1006/jcis.2000.7028
5. Suciati T, Aliyandi AS (2014) Development of transdermal nanoemulsion formulation for simultaneous delivery of protein vaccine and artin-m adjuvant. Int J Pharm Pharm Sci 6(6):536–541
6. Abayomi B, Catherine OE, Enock OD, Sivakumar M, Oluseyi AA, Mopelola AI, Ige AR (2022) Generation, characterization and pharmacokinetic study of ofloxacin-loaded castor oil based nanoemulsion. Acta Chem Malaysia (ACMY) 6(1):20–25. https://www.actachemicamalaysia.com/acmy-01-2022-20-25
7. Escobar-Chávez JJ, Rodríguez-Cruz IM, Domínguez-Delgado CL, Torres R, Revilla-Vázquez AL, Aléncaster NC (2012) Nanocarrier systems for transdermal drug delivery. Skin 8:203–210. https://doi.org/10.5772/50314
8. Guy RH (1996) Current states and future prospects of transdermal drug delivery. Pharm Res 13(12):1765–1769. https://doi.org/10.1023/A:1016066040
9. Unera M, Yener V, Erguven M, Fatma EK, GozdeUtku E (2014) Solid lipid nanoparticles and nanostructured lipid carriers of celecoxib for topical application–preparation, characterization and drug penetration through rat skin. Curr Nanosci 10(4):532–542
10. Tyler VE, Brady LR, Robbers JE (1976) Lipids. Pharmacognosy. Lea and Febiger, Philadelphia, pp 121–122
11. Lee SJ, McClements DJ (2010) Fabrication of protein-stabilized nanoemulsions using a combined homogenization and amphiphilic solvent dissolution/evaporation approach. Food Hydrocoll 24(6):560–569. https://doi.org/10.1016/j.foodhyd.2010.02.002
12. Patel AR (2020) Functional and engineered colloids from edible materials for emerging applications in designing the food of the future. Adv Func Mater 30(18):1806809
13. Lv FF, Li N, Zheng LQ, Tung CH (2006) Studies on the stability of the chloramphenicol in the microemulsion free of alcohols. Eur J Pharm Biopharm 62(3):288–294
14. Yuan JS, Ansari M, Samaan M, Acosta EJ (2008) Linker-based lecithin microemulsions for transdermal delivery of lidocaine. Int J Pharm 349(1):130–143
15. Shakeel F, Baboota S, Ahuja A, Ali J, Aqil M, Shafiq S (2007) Nanoemulsions as vehicles for transdermal delivery of aceclofenac. AAPS PharmSciTech 8(4):191

16. D'Cruz OJ, Uckun FM (2001) Gel-microemulsions as vaginal spermicides and intravaginal drug delivery vehicles. Contraception 64(2):113–123
17. Mishra RK, Soni G, Mishra R (2014) Nanoemulsion: a novel drug delivery tool. Int J Pharm Res Rev 3(7):35–36
18 Podschun R, Ullmann U (1998) *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 11(4):589–603. https://doi.org/10.1128/CMR.11.4.589
19. Bagley ST (1985) Habitat association of Klebsiella species. Infect Control Hosp Epidemiol 6(2):52–58. https://doi.org/10.1017/S0195941700062603
20. Jarvis WR, Munn VP, Highsmith AK, Culver DH, Hughes JM (1985) The epidemiology of nosocomial infections caused by Klebsiella pneumoniae. Infect Control Hosp Epidemiol 6(2):68–74. https://doi.org/10.1017/S0195941700062639
21. Lederman ER, Crum NF (2005) Pyogenic liver abscess with a focus on Klebsiella pneumoniae as a primary pathogen: an emerging disease with unique clinical characteristics. Am J Gastroenterol 100(2):322
22. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL (2012) Carbapenemases in *Klebsiella pneumoniae* and other *Enterobacteriaceae*: an evolving crisis of global dimensions. Clin Microbiol Rev 25(4):682–707. https://doi.org/10.1128/CMR.05035-11
23. Fakhry S, Sorrentini I, Ricca E, De Felice M, Baccigalupi L (2008) Characterisation of spore forming *Bacilli* isolated from the human GI tract. J Appl Microbiol. https://doi.org/10.1111/j.1365-2672.2008.03934.x
24. Salzman NH, de Jong H, Paterson Y, Harmsen HJM, Welling GW, Bos NA (2002) Analysis of 16S libraries of mouse GI microflora reveals a large new group of mouse intestinal bacteria. Microbiology 148:3651–3660. https://doi.org/10.1099/00221287-148-11-3651
25. Hong HA, Khaneja R, Tam NM, Cazzato A, Tan S, Urdaci M, Cutting SM (2009) Bacillus subtilis isolated from the human GI tract. Res Microbiol 160(2):134–143. https://doi.org/10.1016/j.resmic.2008.11.002
26. Casula G, Cutting SM (2002) Bacillus probiotics: spore germination in the GI tract. Appl Environ Microbiol 68:2344–2352. https://doi.org/10.1128/AEM.68.5.2344-2352.2002
27. Tam NMK, Uyen NQ, Hong HA, Duc LH, Hoa TT, Serra CH, Henriques AO, Cutting SM (2006) The intestinal life cycle of Bacillus subtilis and close relatives. J Bacteriol 188:2692–2700. https://doi.org/10.1128/JB.188.7.2692-2700.2006
28. Hauser AR (2009) The type III secretion system of *Pseudomonas aeruginosa*: infection by injection. Nat Rev Microbiol 7:654–665
29. Drlica K, Zhao XK, Zhao X (1997) DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol Mol Biol 61(3):377–392
30. Mehrad B, Clark NM, Zhanel GG, Lynch JP (2015) Antimicrobial resistance in hospital-acquired gram-negative bacterial infections. Chest 147(5):1413–1421. https://doi.org/10.1378/chest.14-2171
31 Fink MP, Snydman DR, Niederman MS, Leeper KV, Johnson RH, Heard SO, Siami GA (1994) Treatment of severe pneumonia in hospitalized patients: results of a multicenter, randomized, double-blind trial comparing intravenous ciprofloxacin with imipenem-cilastatin. The severe pneumonia study group. Antimicrob Agents Chemother 38(3):547–557. https://doi.org/10.1128/AAC.38.3.547
32. Neuhauser MM, Weinstein RA, Rydman R, Danziger LH, Karam G, Quinn JP (2003) Antibiotic resistance among gram-negative bacilli in US intensive care units: implications for fluoroquinolone use. J Am Med Assoc 289(7):885–888. https://doi.org/10.1001/jama.289.7.885
33. Paterson DL, Mulazimoglu L, Casellas JM, Ko WC, Goossens H, Von Gottberg A, Yu VL (2000) Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum β-lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. Clin Infect Dis 30(3):473–478
34. Bamisaye A, Eromosele CO, Dare EO, Akinloye OA, Manickam S, Idowu MA (2020) Development and characterization of PEGylated ofloxacin and ciprofloxacin loaded sandbox oil based nanoemulsion and its potential for transdermal drug delivery. J Chem Soc Nigeria 45(2):265–275
35. Aida P, Rosa V, Blamea F, Thomas A, Salvador C (2001) Parauuan plants used in traditional medicine. Short Commun J Ethnopharmacol 16:93–98
36. National Committee for Clinical Laboratory Standards (NCCLS) (1990) Method for antimicrobial susceptibility testing. Man. Clin Microbiol, 5th Ed.
37. Junaid SA, Olabode AO, Onwuliri FC, Okworiu AEJ, Agina SE (2006) The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial GI isolates. Afr J Biotech 5(22):2315–2321
38. Geo F, Brooks JSB, Stephen AM, Jawetz M (2007) Adelberg's medical microbiology. Sultan Qaboos Univ Med J 22:607
39. Doughari JH, Pukuma MS, De N (2007) Antibacterial effects of *Balanites aegyptiaca* L. Drel. and *Moringa oleifera* Lam. on Salmonella typhi. Afr J Biotechnol 6(19):2212–2215
40. NIH Publication Number 04–4158 (2010) Available at <http://www.win.niddk.nih.gov>.
41. Reitman S, Frankel SA (1957) Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28(1):56–63
42. Tietz NW (1995) Clinical guide to laboratory tests. WB Saunders Co
43. Franco RS, Weiner M, inventors; Franco Robert S (1984) Method for altering red blood cell function and survival. United States patent US 4,478,824
44. Avwioro OG (2002) Histochemistry and tissue pathology, principles and techniques, 1st edn. Claverianum Centre, Ibadan, pp 51–140
45. OECD (2015) Test No. 404: Acute Dermal Irritation/Corrosion, OECD, Publishing, Paris https://doi.org/10.1787/9789264242678-en
46. Wei L, Lu J, Xu H, Patel A, Chen ZS, Chen G (2015) Silver nanoparticles: synthesis, properties, and therapeutic applications. Drug Discov Today 20(5):595–601
47. Yuan G, Dai S, Yin Z, Lu H, Jia R, Xu J (2014) Toxicological assessment of combined lead and cadmium: acute and subchronic toxicity study in rats. Food Chem Toxicol 65:260–268
48. Rašković A, Pavlović N, Kvrgić M, Sudji J, Mitić G, Čapo I, Mikov M (2015) Effects of pharmaceutical formulations containing thyme on carbon tetrachloride-induced liver injury in rats. BMC Complement Altern Med 15(1):442
49. Ajay T, Manpreit K, Harikumar SL (2013) Nanaoemulsionn in enhancement of bioavailability of poorly soluble drugs a review. Pharm Int J 4(1):15–25
50. Ehdaie B (2011) Enhanced delivery of transdermal drugs through human skin with novel carriers. J Pharm Biomed Sci 1(8):161–166
51. Brigger I, Dubernet C, Couvreur P (2012) Nanoparticles in cancer therapy and diagnosis. Adv Drug Deliv Rev 64:24–36
52. Hidron AI, Low CE, Honig EG, Blumberg HM (2009) Emergence of community-acquired meticillin-resistant Staphylococcus aureus strain USA300 as a cause of necrotising community-onset pneumonia. Lancet Infect Dis 9(6):384–392. https://doi.org/10.1016/S1473-3099(09)70133-1
53. Inayat BP, Mallikarjuna S (2012) Nanoemulsion system for transdermal delivery of tamoxifenciterate: design, characterization effect of penetration enhancer and *in vivo* studies. J Nanomat Biostruct 7:1373–1387
54. Al-Adham IS, Ashour H, Al-Kaissi E, Khalil E, Kierans M, Collier PJ (2013) Studies on the kinetics of killing and the proposed mechanism of action of microemulsions against fungi. Int J Pharm 454(1):226–232. https://doi.org/10.1016/j.ijpharm.2013.06.049
55. Ighodaro O, Adeosun A, Adeoye B, Soetan O (2017) Journal of coastal life medicine. J Coastal Life Med 5(4):162–166
56. Saheed S, Oladipipo AE, Abdulazeez AA, Olarewaju SA, Ismaila NO, Emmanuel IA, Fatimah QD, Aisha AY (2015) Toxicological evaluations of Stigma maydis (corn silk) aqueous extract on hematological and lipid parameters in Wistar rats. Toxicol Rep 2:638–644
57. Kumar R, Lal S (2014) Synthesis of organic nanoparticles and their applications in drug delivery and food nanotechnology: a review. J Nanomater Mol Nanotechnol 3:11
58. Gu X, Manautou JE (2012) Molecular mechanisms underlying chemical liver injury. Expert Rev Mol Med 14:e4
59. Anderson ET, Stoskopf MK, Morris JA Jr, Clarke EO, Harms CA (2010) Hematology, plasma biochemistry, and tissue enzyme activities of invasive red lionfish captured off North Carolina, USA. J Aquat Anim Health 22(4):266–273
60. Khan Z, Nawaz M, Khan A, Bacha U (2013) Hemoglobin, red blood cell count, hematocrit and derived parameters for diagnosing anemia in elderly males. Proc Pak Acad Sci 50(3):217–226
61. Olajide JE, Akanji AM, Sanni M, Omale J (2017) Acetylsalicylic acid and cellular damage in kidney of metabisulphite treated rats. Anim Res Int. https://doi.org/10.4314/ari.v2i3.40875
62. Flarer F (1955) The causes of inflammatory erythema. J Invest Dermatol 24:201–209

63. Draize JH (1944) Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 82:377–390
64. Ramanunny AK, Wadhwa S, Singh SK, Kumar B, Gulati M, Kumar A, Chellappan DK (2022) Topical non-aqueous nanoemulsion of *Alpinia galanga* extract for effective treatment in psoriasis: in vitro and in vivo evaluation. Int J Pharm 624:121882
65. Harwansh RK, Patra KC, Pareta SK, Singh J, Rahman MA (2011) Nanoemulsions as vehicles for transdermal delivery of glycyrrhizin. Braz J Pharm Sci 47(4):769–778
66 Barradas TN, De Holanda Silva KG (2021) Nanoemulsions of essential oils to improve solubility, stability and permeability: a review. Environ Chem Lett 19(2):1153–1171
67. Iqbal R, Mehmood Z, Baig A, Khalid N (2020) Formulation and characterization of food grade O/W nanoemulsions encapsulating quercetin and curcumin: insights on enhancing solubility characteristics. Food Bioprod Process 123:304–311

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