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Original Research Article

Study the Effective Role of Metronidazole Nanoemulsion for the Treatment of Skin Lesions in Mice Induced by *Entamoeba Histolytica*

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Abstract: The current study aimed to create a metronidazole nanoemulsion and then use it in the treatment of skin lesions in mice caused by *Entamoeba histolytica*. We used parasite isolates which taken from the Department of Biology, College of Science, University of Kirkuk. It was rediagnosed by conventional methods. After prepared metronidazole nanoemulsion. We selected 24 healthy males Mus musculus with an average weight of 20-25 grams mice aged 9-12 weeks from, the animals were randomly divided into four groups, with six animals in each group, as follows Control group which injected with normal saline in a volume of 0.1 ml. The second group was injected with a suspension of E. *histolytica* in a volume of 0.1 ml and at a concentration of 1 x 10^8 CFU. The third group was injected with Mt-nanoemulsion 0.1 ml. The fourth group was injected with a suspension of *E. histolytica* and treated with Mt-nanoemulsion. The solubility investigation was conducted using a variety of vehicles to determine the optimal solvent for isradipine's dissolution. Pseudo ternary phase diagrams are created at surfactant and co-surfactant mix related ratios of (1:1, 1:2, 1:3, 1:4, and 2:1). Eight nano emulsions were created by combining several amounts of (Transcutol, Tween20, and Triacetin). The findings indicated that Triacetin was included in the formulations to preserve the medication in a solubilized state and prevent precipitation of the drug because Triacetin had a better solubility of metronidazole. The results of the in vitro study showed that the levels of Malondialdehyde were substantially greater ($P < 0.05$) in the *E. histolytica* group than in the normal mouse group. Glutathione and catalase levels in the *E. histolytica* group were substantially lower ($P<0.05$) than those of normal mice. MDA, GSH, and catalase levels did not differ significantly between the Mt-nanoemulsion-treated group and the control group ($P>0.05$).

Keywords: *Entamoeba histolytica*, metronidazole, nanoemulsion, Amebiasis.

INTRODUCTION

Entamoeba histolytica the most common intestinal protozoan parasite which causes Amebiasis [1]. It is infected the large intestinal area causing intestinal colic, diarrhea occasionally diarrhea with bleeding of mucosa, ulceration in the walls of the large intestine, colon wall invasion or the growth of non-pathogenic colonies in the bowels [2], and spread dysentery worldwide [3]. The mortality rate with *Entamoeba histolytica* was recorded over 100000 worldwide yearly [4-5]. About 10% of cases are asymptomatic, although the parasite can invade the gut wall causing sever ulceration and amoebic dysentery characterized by blood [6]. The parasite transmation through contaminated food and water via fecal–oral route [7].

Thermodynamically stable transparent or translucent dispersions of oil and water known as nanoemulsions, sub-micron emulsions, or mini-emulsions are held together by an interfacial coating of surfactant and cosurfactant molecules with globule sizes of less than 100 nm [8]. Nanoemulsions are currently utilized extensively for the administration of vaccines, DNA-encoded drugs, antibiotics, cosmetic and topical preparations, and are administered by a variety of routes including oral, pulmonary, intranasal, ophthalmic, and transdermal [9-11]. As a type of multiphase colloidal dispersion,

nanoemulsions are distinguished by their stability and clarity. Little particles or droplets that have very little oil/water interfacial tension often make up the dispersed phase [12]. Nanoemulsions occur spontaneously, easily, and occasionally without the need for high energy input. A cosurfactant or cosolvent is often used in addition to the surfactant, oil phase, and water phase [13-16]. The best drug delivery methods are nano-emulsions because they can dissolve large amounts of poorly soluble medications, are generally compatible, and can shield drugs from enzymatic and hydrolytic breakdown [17]. The goal of the current investigation was to treat mice with skin lesions brought on by *E. histolytica* using a metronidazole nanoemulsion that was made.

MATERIALS & METHODS

Materials

We purchased metronidazole, castor oil, sun flower oil, cinnamon oil, triacetin, Tween 20 and Tween 80, ethanol, and methanol from the local market in Kirkuk, Iraq. All of the trials we used distilled water.

Solubility Studies

Metronidazole solubility's in Castor oil, co-surfactants, and surfactants were calculated. In a screw-capped glass tube, 2 mL of the excipient were combined with extra metronidazole. To achieve equilibrium, the mixture was agitated by an isothermal shaker at 25 C^{o} for 48 h. The drug concentration was then determined by HPLC after each tube had been spun at 5000 rpm for 15 min. The supernatant had then been diluted with methanol.

Construction of Pseudo Ternary Phase Diagrams

Oil, a mixture of surfactant and co-surfactant and water were produced by using water titration technique to generated components for pseudo ternary phase diagrams [18]. Different ratios of co-surfactant and surfactant were combined (1:1, 1:2, 1:3, 1:4, 2:1). According to each phase diagram, oil and surfactant mix were combined in a range of weight ratios until the maximum ratio of surfactant mix and oil was reached. These mixtures were then gradually titrated with water (aqueous phase), with each mixture being titrated by using a gentle magnetic stirrer. The titration's endpoint was determined by measuring the water concentration at which transparent-to-turbid shifts took place [19].

Centrifugation Test

During 20 to 30 minutes, all of the resulting nanoemulsion formulations were centrifuged at 3500 rpm to check for cracking, creaming, and phase separation.

Heating-Cooling Cycle (H/C Cycle)

Six cycles (4–45° C) are used, with 48 hours of storage at each temperature. Formulations that are thought to be stable at these temperatures underwent a cycle of freezing and thawing.

Zeta Potential Measurement

Zeta potential is explicable A parameter is used to assess the characteristics of surface charge and to indicate the a physical stability of nanoemulsions [20].

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was used to assess the medication and formulation's compatibility. The spectrum for was acquired. It is utilized to determine the functional groups' modes of attachment and the molecule's unique fingerprint. The sample can be prepared using a suitable technique, such as the Nujol mulls, and then scanned in FTIR at a moderate scanning speed of (4000 to 400 cm^{-1}) [21].

Field Emission Scanning Electron Microscope (FESEM)

Field emission scanning electron microscopy analysis of metronidazole nanoemulsions was conducted (FESEM). It is a device that is utilized for an image surface roughness analysis in order to explain the size and shape of the droplets included within the created metronidazole nanoemulsion [22].

Entamoeba Histolytica Isolates

The isolates of *E.histolytica* used were from various clinical samples. These isolates were obtained in September 2022 from the Biology Department of the College of Science at the University of Kirkuk. It was rediagnosed utilizing both traditional techniques and API 20 E systems.

Injection Method

Once the hair was clipped off the animal's back and contaminated with an *E. histolytica* suspension, a transcutaneous wound (10 mm long) was created. The mice groups were dissected three days after the injection, and pieces of the damaged skin were removed and put in a fixative of formalin (10%) for 24 hours. Depending on the approach used by [23] the tissue sections were then processed for examination of the pathogenic consequences after an hour.

Study Animals

24 healthy male Mus musculus mice, aged 9 to 12 weeks, with an average weight of 20 to 25 grams, were chosen from the Experimental Research Unit/Biotechnology/Al-Nahrain University and housed in metal cages which was cleaned with sawdust, and changed every three days for maintaining the cages cleanliness Animals were kept in identical laboratory settings throughout the duration of the trial, with temperatures between 20 and 26 C and consistent airflow. The animals were given a diet that included concentrated feed as its main ingredient. With six animals in each group, the animals were separated into four groups at random as follows:

- Control group: was injected with normal saline in a volume of 0.1 ml
- The second group: was injected with a suspension of E. *histolytica* in a volume of 0.1 ml and at a concentration of 1 x 10^8 CFU.
- The third group: was injected Mt-nanoemulsion 0.1 ml.
- The fourth group: was injected with a suspension of E. *histolytica* and treated with Mt-nanoemulsion.

Statistical Analysis

The average of three readings from each sample are included in the study's findings. In order to determine whether study results were regarded significant when ($P < 0.05$) and not significant when ($P > 0.05$), the analysis of variance test (ANOVA) was used [24].

RESULTS & DISCUSSION

Saturation Solubility Study of Metronidazole

Because Triacetin had a better solubility than other solvents, it was utilized in formulations to keep the medication solubilized and prevent drug precipitation, as indicated in table (1) [25]. Tween 80 was chosen as a surfactant for obtaining a one phase clear solution [26]. In the presence of co-surfactants, ethanol was found to have a higher solubilizing capacity for metronidazole, this discovery would increase the miscibility of the water soluble and oily so that to partitioning between these phases, reducing interfacial tension and increasing the mobility of the hydrocarbon tail, allowing more oil to penetrate into this area [27]

Table 1: Solubility study of metronidazole in different oils, surfactants and co- surfactants

Oil	**Solubility (mg/ml)**
Castor oil	12.34±0.192
Sun flower oil	4.517±0.01
Triacetin	38.391±0.082
Cinnamon oil	1.805±0.017
Surfactant	**Solubility (mg/ml)**
Tween 20	19.45±0.031
Tween 80	42.951±0.047
Co surfactant	**Solubility (mg/ml)**
Ethanol	51.371±0.281
Methanol	21.206±0.315

Evaluation of the Selected Metronidazole Optimum Formula Drug and Excipient Compatibility Study By FTIR

While making NEs, the FTIR approach is incredibly effective at identifying and assessing any potential chemical interactions between metronidazole and any excipient. Pure metronidazole powder's FTIR spectra revealed the following characteristic peaks 3348.78 cm^{-1} due to (N-H) stretching vibration, 3109.65-2976.59 cm^{-1} corresponding to (=C-H) stretching, 2930.31 cm^{-1} due to aliphatic (C-H) stretching, 1674.87 cm^{-1} for ester (C=O) stretching, 1495.53 cm^{-1} and 1451.17 cm^{-1} due to aromatic C of the FTIR spectrum of pure metronidazole and the chosen formula both showed the same functional group band with only very slight shifting, which is consistent with hydrogen bonding [28].

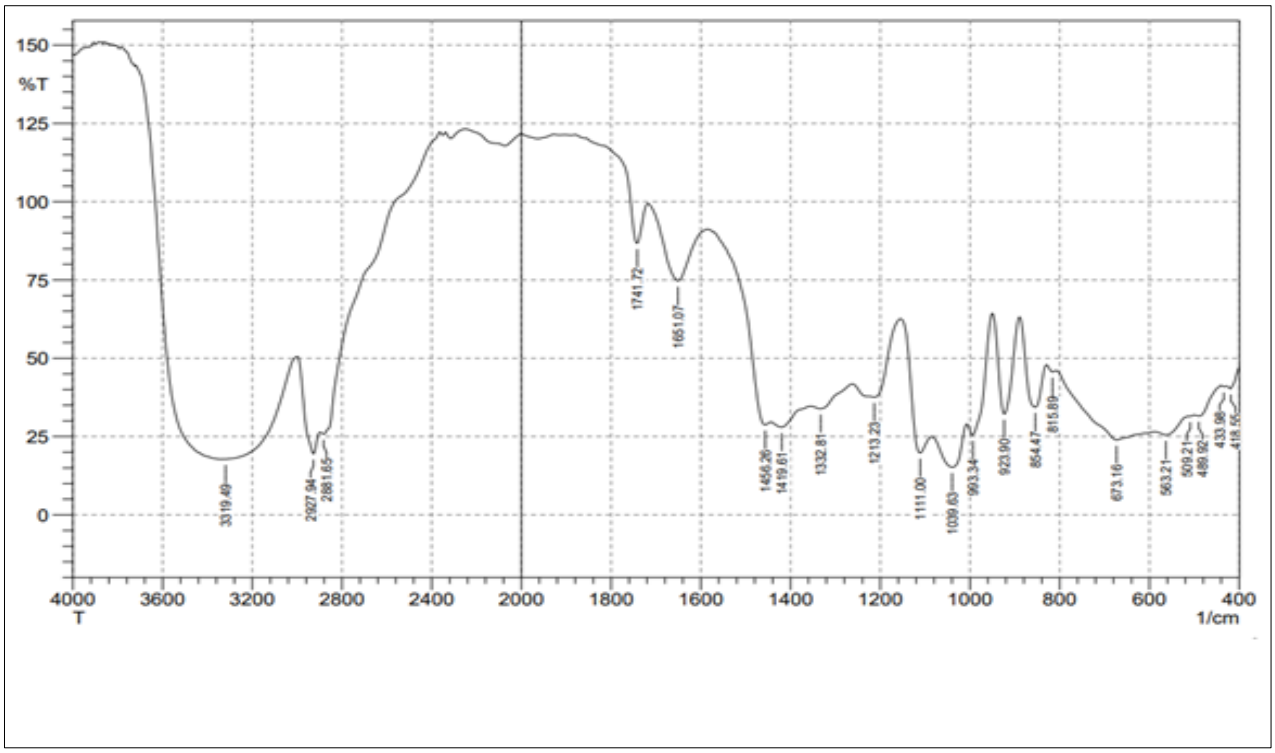

Figure 1: FTIR spectrum of metronidazole

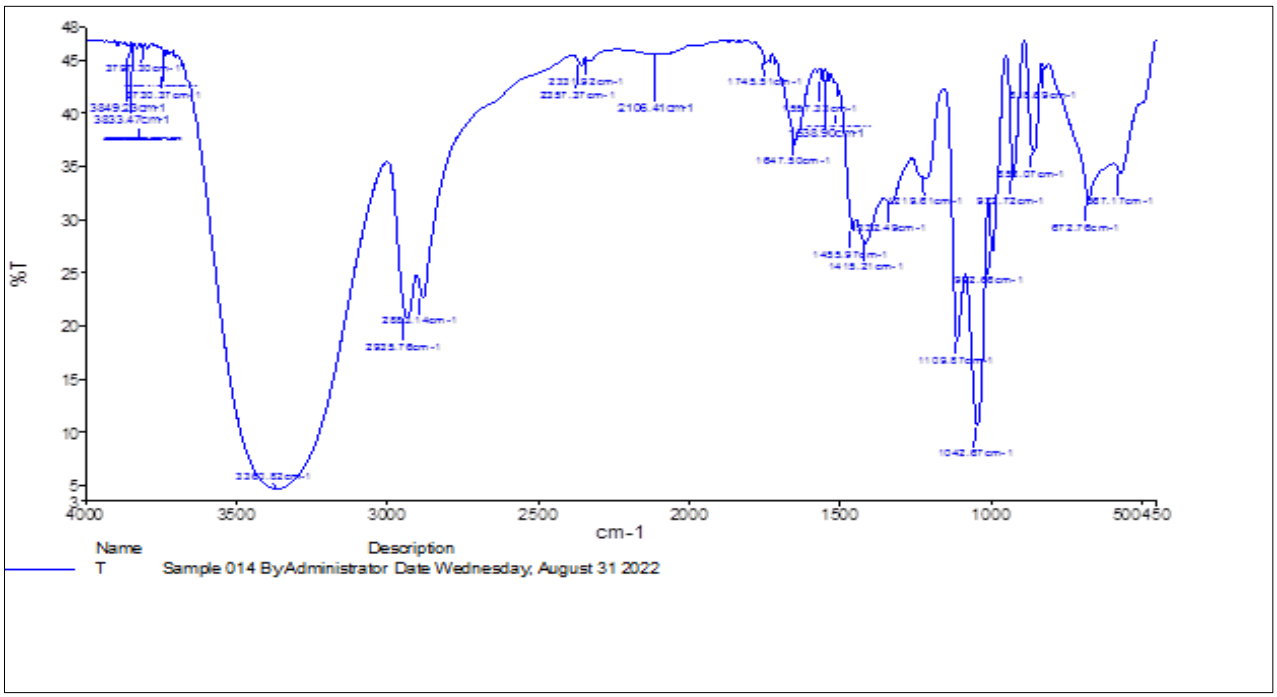

Figure 2: FTIR spectrum of the selected formula

FESEM

Figure 3 shows that the optimized formula's oil droplets are spherical in shape and accumulate the smaller oil droplets, but there are no significant changes in the size or shape of the emulsion droplets upon accumulation [29].

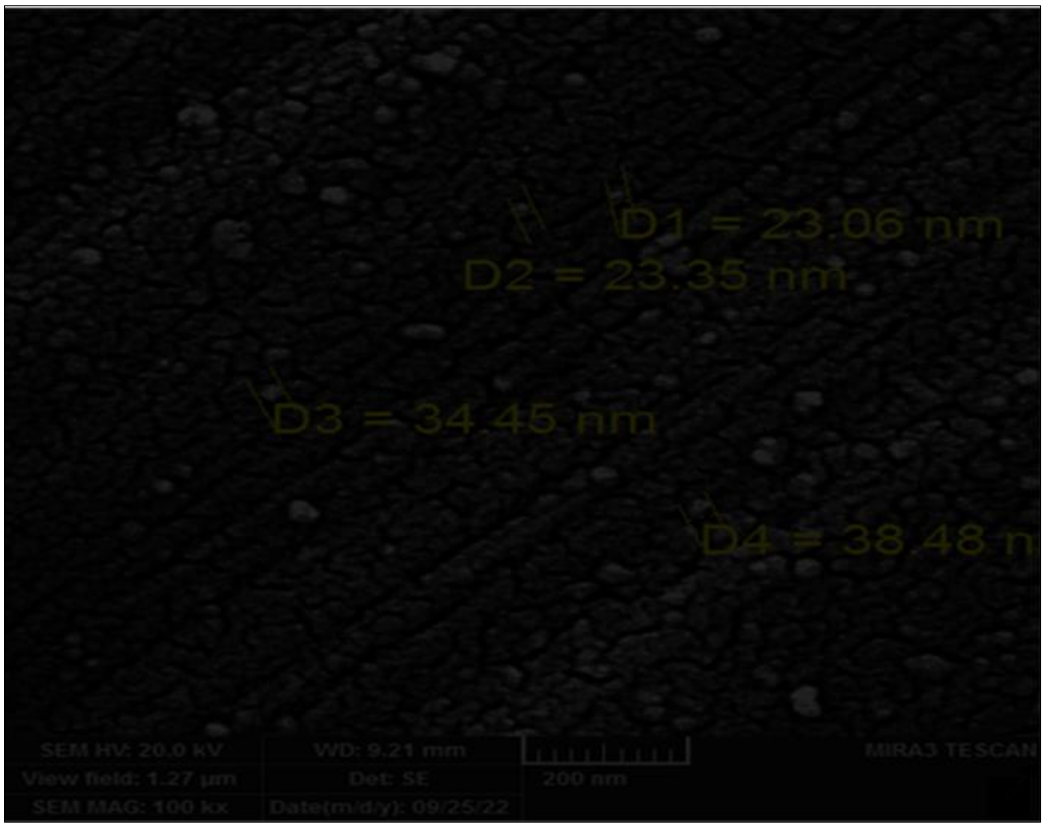

Figure 3: FESEM of optimized formula

In Vitro Study

Induction of Infection

The infection of cutaneous amebiasis was developed, as described in the method of work, where the infection area was shaved, then incisions were made in the skin, and the area was wiped with parasitic saline solution, as in the figure 4.

Figure 4: Induced the infection of cutaneous amebiasis

Oxidative Status

The results of this investigation were shown in Table 2, which showed that the MDA levels in the *E. histolytica* group were substantially higher ($P<0.05$) than other groups. GSH and catalase levels in the *E. histolytica* group were substantially lower ($P< 0.05$) than other groups. MDA, GSH, and catalase levels did not differ significantly between the Mt-nanoemulsion-treated group and the control group ($P>0.05$).

Table 2: Levels of MDA, GSH and catalase in all groups

Parameters / Groups	MDA (umol/l)	GSH (umol/l)	Catalase (umol/l)
Group A	1.493±0.17	0.358±0.027	1.38±0.17
Group B	2.804±0.47*	0.207±0.031*	0.73±0.05*
Group C	1.367±0.08	0.362±0.019	1.45±0.13
Group D	1.692±0.12	0.322±0.042	1.29±0.03
P value	0.0001	0.001	0.001

According to Al-Kaky [30], patients with E. *histolytica* have higher levels of MDA and lower levels of GSH when compared to the control group. The levels of oxidative stress and antioxidants show significant changes [31]. Propose that the ability of the parasite to produce free radicals, which promote cytological alterations, is the cause of the elevated MDA levels and decreased GSH. Antiparasitic medicines bioactivity and stability can both be improved with the use of micro- and nanoemulsions [32]. Studies conducted in-vivo on infected mice revealed that treatment with nanoemulsion considerably reduced the size and number of the largest cysts when compared to treatment with un emulsified oil [33]. In terms of the potential of metronidazole nanoemulsion to treat *Entamoeba histolytica* infection, this validates the findings of the present investigation.

REFERENCES

1. Garcia, L. S., Shimizu, R. Y., Novak, S., Carroll, M., & Chan, F. (2003). Commercial assay for detection of Giardia lamblia and Cryptosporidium parvum antigens in human fecal specimens by rapid solid-phase qualitative immunochromatography. *J Clin Microbiol*, *41*, 209–12.
2. Al-Abodi, H. R. J. (2018a). Pathological Effects Associated with Parasitic Infections. *Indian Journal of Natural Sciences*, *9*(51), 16146-16151.
3. Shaker, E. M., Al-Shaibani, K. T. M., & Al-abodi, H. R. J. (2018). Effect of Alcohol extract of green tea plant Camellia sienensis as a therapeutic of parasite Entamoeba histolytica. *Plant Archives*, *18*(1), 953-959.
4. Al-Abodi, H. R. J. (2018b). Suspicion in the form of infection is the basis for selecting the appropriate method for examining the toxoplasmosis disease of bends that have no symptoms from patients. *Int. J. Adv. Res.*, *6*(9), 655-662.
5. Hamzah, Z., Petmiter, S., Mangthin, M., Leelayoova, S., & Chavalitsh, P. (2006). differential detection of Entamoeba histologica, Entamoeba dispar and E.Moshicoviskiiby single round PCR assay. *J. clin. microbial.*, *4*, 3196 -3200.
6. Debbie-Ann, T. S., Laura F., Koji, W., & Shannon M. (2018). A Review of the Global Burden, New Diagnostics, and Current Therapeutics for Amebiasis. *Open Forum Infectious Diseases*, *5*(7), 161-167.
7. Ali, O. S., Shihab, A. M., & Yahya J. S. (2018). Relationship between Entamoeba histolytica and Fecal Calprotectin in Patients with Gastroenteritis in Kirkuk City-Iraq. *EJMM*, *27*(2), 49-56.
8. Shahad, A. A. Al-Attar & Abeer, A. A. (2020). Phenolic Compounds Role in Rat Immunity Changes that Caused by Entamoeba Histolytica. *Medico Legal Update*, *20*(2), 497–501.
9. Fotedar, R. D., Beebe, N. D., Ellis, M. J., & Harkness, J. (2011). PCR detection of Entamoeba histolytica, Entamoeba dispar and Entamoeba moshkovskii in stool samples from Sydney, Australia. *J. Clin. Microbio*, *45*, 1035–1037.
10. Abhyankar, M. M., Orr, M. T., Kinsey, R., Sivananthan, S., Nafziger, A. J., Oakland, D. N., Young, M. K., Farr, L., Uddin, M. J., & Leslie, J. L. (2021). Optimizing a multi-component intranasal Entamoeba histolytica vaccine formulation using a design of experiments strategy. *Front. Immunol.*, *12*, 683157.
11. Jasni, N., Saidin, S., Kin, W. W., Arifin, N., & Othman, N. (2022). Entamoeba histolytica: Membrane and Non-Membrane Protein Structure, Function, Immune Response Interaction, and Vaccine Development. *Membranes*, *12*, 1079.
12. Thakur. N., Garg, G., Sharma, P. K., & Kumar, N. (2012). Nanoemulsions: A Review on Various Pharmaceutical Applications. *Global Journal of Pharmacology*, *6*(3), 222-225.
13. Thiagarajan, P. (2011). Nanoemulsions for drug delivery through different routes. *Research in biotechnology*, *2*(3).
14. Sharma, N., Bansal, M., & Visht, S. (2010). Nanoemulsion: A new concept of delivery application of nanoemulsion. *System.*, *1*(2), 2-6.
15. Devarajan, V., & Ravichandran, V. (2011). Nanoemulsions: As Modified Drug Delivery Tool. *International Journal of Comprehensive Pharmacy*, *4*(01), 1-6.
16. Shah, P., & Bhalodia, D. (2010). A Pharmaceutical Review. *Sys Rev Pharm*, *1*(1), 24-32.
17. Ravi, T. P., & Padma, T. (2011). Nanoemulsions for drug delivery through different routes. *Res. Biotechnol.*, *2*(3), 1- 13.
18. Schalbartm, M., & Kawaji, K. (2010). Formation of tetradecane nanoemulsion by low-energy emulsification methods. *International journal of refrigeration*, *33*, 1612-1624.
19. Ghareeb, M. M., & Neamah, A. J. (2017). Formulation and characterization of nimodipine nanoemulsion as ampoule for oral route. *International Journal of Pharmaceutical Sciences and Research*, *8*(2), 591.

20. Manyarara, T. E., Khoza S., Dube, A., & Maponga, C. C. (2018). Formulation and characterization of a paediatric nanoemulsion dosage form with modified oral drug delivery system for improved dissolution rate of nevirapine. *MRS Advances, 3*(37), 2203-19.
21. Kumar, R., Soni, G., & Prajapati, S. K. (2017). Formulation development and evaluation of Telmisartan Nanoemulsion. *IJRDPL, 6*, 2711-9.
22. Affandi, M. M., Julianto, T., & Majeed, A. (2011). Development and stability evaluation of astaxanthin nanoemulsion. *Asian J Pharm Clin Res, 4*(1), 142-8.
23. Suvarna, M., Niranjan, U. C., & Karjodkar, F. R. (2013). Classification methods of skin burn images. *International Journal of Computer Science & Information Technology, 5*(1), 109.
24. Shaw, R. G., & Mitchell, T. (1993). ANOVA for unbalanced data: an overview. *Ecology, 74*(6), 1638-1645.
25. Saleh, A. H. (2019). Potential effect of green zinc oxide nanoparticles in treatment of kidney lesions that induced by Burkholderia mallei in albino male rats. *Biochemical and Cellular Arch., 19*, 2439–2443.
26. Subongkot, T., & Ngawhirunpat, T. (2017). Development of a novel microemulsion for oral absorption enhancement of all-trans retinoic acid. *Int J Nanomedicine, 12*, 5585–99.
27. Chatterjee, B., Hamed, A. S., Ahmed, M. D. A., & Mandal, U. (2016). Controversies with self-emulsifying drug delivery system from pharmacokinetic point of view. *Drug Deliv, 23*(9), 3639–52.
28. Kesan, K., Minyak, F., & Terhadap, S. (2018). Study on the Effect of Oil Phase and Co Surfactant on Microemulsion Systems. *Malaysian J Anal Sci, 21*(6), 1409–16.
29. Sandeep, V., Narendar, D., Arjun, N., & Kishan, V. (2016). Lacidipine loaded solid lipid nanoparticles for oral delivery: preparation, characterization and in vivo evaluation. *Int. J. Pharm. Sci. Nanotechnol, 9*(6), 3524–30.
30. Affandi, M. M., Julianto, T., & Majeed, A. (2011). Development and stability evaluation of astaxanthin nanoemulsion. *Asian J Pharm Clin Res., 4*(1), 142-8.
31. Al-Kaky, I. S. (2006). Lipid peroxidation and some hematological changes in Entamoeba histolytica patients. *Raf. J. Sci., 17*(9), 10-16.
32. Echeverría, J., de Albuquerque, D. G., & Diego, R. (2019). Nanoemulsions of essential oils: New tool for control ofvector–borne diseases andin vitroeffects on some parasitic agents. *Medicines, 6*, 42.
33. Moazeni, M., Borji, H., Darbandi, M. S., & Saharkhiz, M. J. (2017). In vitroandin vivoantihydatid activity of a nanoemulsion of Zataria multiflora essential oil. *Res. Vet. Sci, 114*, 308–312.