Original Article

Date Seed Extract-loaded Oil-in-Water Nanoemulsion: Development, Characterization, and Antioxidant Activity as a Delivery Model for Rheumatoid Arthritis

Abdul Qadir1†, Mohd Aqil2, Usama Ahmad1, Nausheen Khan3, Musarrat H. Warsi4, Juber Akhtar1, Muhammad Arif5, Abuzer Ali5, Satya P. Singh1

1Department of Pharmaceutics, Faculty of Pharmacy, Integral University, Lucknow, Uttar Pradesh, India, 2Department of Pharmaceutics, School of Pharmaceutical Education & Research Jamia Hamdard, New Delhi, India, 3Department of Pharmacognosy and Phytochemistry, School of Pharmaceutical Education & Research Jamia Hamdard, New Delhi, India, 4Department of Pharmaceutics and Industrial Pharmacy, College of Pharmacy, Taif University, Taif-Al-Haweiah, Saudi Arabia, 5Department of Pharmacognosy, College of Pharmacy, Taif University, Taif-Al-Haweiah, Saudi Arabia, †Present address: Department of Pharmaceutics, School of Pharmaceutical Education & Research Jamia Hamdard, New Delhi, India

Rheumatoid arthritis is an inflammatory disorder, affecting around 1% of the world population. Antioxidant activity plays an important role to overcome the inflammation associated with arthritis. Phoenix dactylifera (date) seeds, generally considered as a waste product or utilized as food for domestic farm animals, have been used as a source of antioxidants at different disease conditions. The aim of the present study was to enhance the release of date seed extract in order to achieve high antioxidant activity. Nanoemulsion of methanolic extract of date seed was prepared by aqueous titration method. The selected formulations were exposed to thermodynamic stability and dispersibility tests. The optimized nanoemulsions were evaluated on the basis of droplet size (23.14 ± 0.055 nm), polydispersity index (0.166 ± 0.124), percent transmittance (99.12 ± 0.0163), refractive index (1.36 ± 0.046), viscosity (cP) (12.30 ± 0.75), conductivity (µS/cm) (347.46 ± 1.10), and drug content (%) (99.67 ± 0.11). The in vitro release studies revealed that final optimized formulation has cumulative release of drug (57.51% ± 2.65%), which was more significantly greater as compared to drug suspension (26.44% ± 1.15%). Further in vitro antioxidant activity studies revealed that the developed methanolic extract of date seed-loaded nanoemulsion has more antioxidant potential when compared with methanolic extract.

KEYWORDS: Antioxidant, nanoemulsion, Phoenix dactylifera, rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is a most common inflammatory disorder. It is portrayed at the cell...
level by changes in the natural and adaptive immunity, which creates a constant synovial joint provocative reaction that may influence other organs.[3] Studies in support of RA have given the proof that RA is a dynamic insusceptible cell-intervened illness, which is started and advanced by variant actuation of T cells with related B-cell hyperactivity. The deregulation of invulnerable cell action causes incessant synovial joint inflammation that is considered fundamentally by T-cell-interceded actuation of synovial fibroblasts.[2] This drives the dynamic obliteration of cartilage and subchondral bone bringing about joint disaster.[9] Free radicals are involved in constant incendiary illnesses including RA and some other conditions. Free radicals assume a significant job in the seriousness of rheumatoid joint pain and patients, for the most part, endure with excessive oxidative stress. Antioxidants either engineered or natural are intense foragers of free radicals and effectively affect human well-being and illness avoidance.[8] It has been reported that antioxidants have plausible role for uplifting the inflammatory condition in RA patients.[5] Several literature reported that synthetic antioxidants (butylated hydroxyanisole, butylated hydroxytoluene) may be the cause of cancer development. So, natural antioxidants are extremely desirable.[6]

Phoenix dactylifera (date palm) belongs to the family Palmaceae and genus Phoenix, cultivated globally, especially in Middle East and North Africa for its palatable organic nature. A date is a high-vitality nourishment having high value of carbohydrates (70%–80%) and low fat content, and is a great wellspring of phenolic compounds, vitamins, carotenoids, zinc, iron, calcium, magnesium, phosphorous, and potassium. Date seeds are generally considered as a waste item and either disposed of or utilized in as animal feed. Nevertheless, date seeds have been appeared to have highly extractable valuable components.[7] The preventive effects of natural antioxidants in leafy foods are associated with four significant groups of compounds such as polyphenols, alkaloids, vitamins, and carotenoids.

Edible portion of date and date seed has antioxidant activity in vitro because of the existence of phenolic compounds.[9] Defensive effect of phenolic compounds as antioxidants is related to lipoperoxidative impairment, which relies on the hydrogen donating limit of hydroxyl bunch in every molecule.[9]

The nanotechnology-based drug delivery system such as nanomicelles, nanoemulsions, nanosuspension, nanoparticles, and some other nanoformulations has displayed a promising value in enhancing the delivery of hydrophobic and hydrophilic molecules since past decades.[9] Nanoemulsion provides very low interfacial tension with substantial oil-in-water interfacial regions, high dynamic strength, and optical transparency approximating to microemulsion.[11,12] Nanoemulsions offer a higher solubilization limit than a straightforward micelle arrangement and their thermodynamic steadiness offers favorable circumstances over flimsy scatterings, for example, emulsion and suspension.[13] Nanoemulsions may also be employed as an alternate for lipidic nanocarriers (liposomes and vesicles) to enhance bioavailability of many active biomolecules when compared with the traditional formulations.[14] Thus, to improve the antioxidant activity of date seed, a robust and stable nanoemulsion system containing date seed extract was developed and optimized. The developed nanoemulsions were further evaluated for mean droplet size and its surface morphology, viscosity, conductivity, refractive index (RI), in vitro drug release, and in vitro antioxidant activity.

Materials and methods

Materials and chemicals
Date seeds were purchased from local in a full ripe condition and authenticated by Dr. Y. T. Kamal, Assistant Professor, Department of Pharmacognosy and Phytochemistry, College of Pharmacy, Sattam Bin Abdul Aziz University, KSA (accession no. PSA/PHAR/COG/15/04). Sefsol 218 and Kolliphor RH40 were acquired as a bequest from Nikko Chemicals (Tokyo, Japan) and BASF (Mumbai, India), respectively. PEG 400 was procured from S.D. Fine-Chem (India). All chemicals used were of analytical grade like Isopropyl myristate (IPM), Olive oil, Methanol (HPLC grade) were purchased from Merck (Mumbai).

Methodology

Methanolic extract of date seeds
Date seeds (200 g) were extracted with 500 mL of methanol using Soxhlet apparatus for 24 h at 50°C–60°C. After extraction, the solvent was evaporated to dryness using a rotary vacuum evaporator (Hahn SHIN, HS-2005 V-N) at 40°C under an inert atmosphere to obtain a dark brown-colored residue. This obtained material was weighed and percentage yield was calculated.[13]

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\text{Percent yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100
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Nanoformulation development and its characterization
To achieve stable and robust nanoemulsion, a through screening of various components was done. For this,
solubility studies of methanolic extract of date seed were performed with different oils, surfactants, and cosurfactants. Afterward, construction of pseudoternary phase diagram was carried out followed by testing of their thermodynamic stability of developed nanoemulsions.

**Screening of oil, surfactants, and cosurfactants by solubility studies**

An important criterion for a stable nanoemulsion is to find out the solubility of a drug in selected oils. In this sequence, solubility of date seed extract was analyzed in different oils (Sefsol 218, Capryol 90, oleic acid, and isopropyl myristate [IPM]). An excess quantity of date seed extract was poured in stoppered vials containing 2 mL of each oil and blended utilizing a vortex.\(^2\) To get equilibrium, these vials were further kept at 25°C ± 1.0°C in an isothermal shaker (IKA KS 400i, Germany) for 72 h. Afterward, these vials were centrifuged (REMICM are -8 Plus and CM-12 Plus, India) at 10,000 rpm for 15 min. The supernatant obtained was filtered over a 0.45-μm membrane filter and the quantity of date seed extract was further estimated in various oils by a UV spectrophotometer (UV-1800, Schimadzu) at a wavelength of 280 nm. A qualitative solubility test was performed for selecting a surfactant and cosurfactant. Final selection was done based on the miscibility of extract loaded oil with surfactants.

**Preparation of nanoemulsion**

Nanoemulsions were formulated by aqueous titration method followed by construction of pseudoternary phase diagrams to determine the concentration range of oil, \(S_{\text{mix}}\) (surfactant–cosurfactant mixture), and distilled water.\(^2\) Different \(S_{\text{mix}}\) ratios were prepared by mixing surfactants and cosurfactants in different quantities (1:0, 1:1, 1:2, 2:1), one or the other in increasing concentration with respect to each other. For every phase diagram, oil and a definite \(S_{\text{mix}}\) ratio were blended properly in distinct volume ratios from 1:9 to 8:1 in individual glass vials. Then the blend (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 1:2, 1:3, 1:3.5, 1:5, 1:6, 1:7, and 1:8) was titrated slowly with aqueous phase. Each titration was visually examined for transparency and flowability. The physical condition of the nanoemulsion was set apart on the phase diagrams with three pivots demonstrating an aqueous phase, oil, and \(S_{\text{mix}}\). For each stage graph, nanoemulsion territory was drawn and the more extensive district showed the improved self-nanoemulsifying proficiency. From every phase diagram, constructed, various formulations were selected from nanoemulsion region altering the proportion of oil (10-30%v/v) at minimum concentration of \(S_{\text{mix}}\). Furthermore, the selected nanoemulsions were studied for stability and dispersibility.

**Thermodynamic stress stability studies**

Designated nanoemulsions were analyzed for the stress stability tests (heating–cooling cycle, centrifugation, and freeze–thaw cycle):

- Heating–cooling cycle: Formulations were exposed to 45°C and 0°C for 48 h for each temperature round and examined for any type of precipitation or phase separation.
- Centrifugation study: Selected nanoemulsions were further centrifuged at 5000 rpm (REMI) for 30 min and diagnosed for any possible non-homogeneity of formulations.
- Freeze–thaw cycle: In this study, nanoemulsions were maintained at the temperatures −20°C and +20°C for three cycles and each cycle for 24 h and these formulations were observed for homogeneity.\(^6\)

**Dispersibility tests**

Every selected formulation (1 mL) was gently added to a medium of 0.1 N HCl (500 mL) in an USP Type II dissolution apparatus (75 rpm) at 37°C ± 0.5°C, in order to evaluate its proficiency of self-emulsification.\(^7\) All formulations were observed visually as per the grading system given in Table 1.

Formulations that agreed with the stability and dispersibility criteria were selected for preparation of date seed extract-loaded nanoemulsion followed by further characterization.

**Preparation of drug-loaded nanoemulsion by aqueous titration method**

The drug-loaded nanoemulsion (DLN) was fabricated by dissolving 50 mg (single dose)\(^8\) of methanolic date seed extract in oil (5%, 10%, 15%, 20% vol/vol). Respective \(S_{\text{mix}}\) ratio was mixed with oil using vortex followed by gently adding aqueous phase to achieve nanoemulsion.

**Physicochemical characterization and evaluation of nanoformulation**

**Visual inspection**

Visual inspection was performed to distinguish the drug-loaded nanoemulsion and macroemulsion.

**Droplet size measurement**

The average droplet size of nanoemulsions was established by dynamic light scattering method with

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**Table 1: Dispersibility test**

| Grade | Appearance of emulsion | Remark               |
|-------|------------------------|----------------------|
| A     | Clear                  | Rapidly form within a minute |
| B     | Slight less clear      | Rapidly forming      |
| C     | Fine milky emulsion    | Form in 2 min        |
| D     | Insipid white emulsion | Slow emulsification (>2 min) |
| E     | Presence of bigger oil globules | Poor emulsification |
the help of zetasizer ZS 90 (Malvern Instruments, Worcestershire, UK). Scattering of light was done at the room temperature at a 90° angle. Polydispersity index (PDI) of the formulations was also recorded.[16]

**Viscosity measurement**

Viscosity of the developed nanoemulsions was also determined by a Brookfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratories, Middleboro, Massachusetts) with spindle CPE40 at 25°C ± 0.5°C.

**Surface morphology**

Surface morphology of the developed formulation was explored by transmission electron microscopy (TEM; TOPOCON 002B, Topcon). One drop of the selected formulation was taken and attenuated with distilled water (1:100); after filtering with a syringe filter (0.22 μm), the sample was placed on the carbon grid with 2% phosphotungstic acid and allowed to stay for 30 s. This dried grid was placed on a slide and covered up with a cover slip. The prepared slide was analyzed using the microscope.

**Drug content**

There is a possibility of precipitation of active drug due to the presence of surfactant and cosurfactant in the formulation by the centrifugation method. Hence, the amount of drug in the developed formulations was calculated by using UV spectroscopy. The selected formulations were diluted with methanol and the absorbance was recorded at 280 nm. Drug content was expressed as a percentage of date seed extract determined in the formulation to the theoretical quantity of the drug added.

**In vitro drug release profile**

The optimized nanoformulations were further investigated for the drug release profile by the dialysis bag method. Dialysis membrane was preactivated in a solvent system of phosphate buffer (pH 7.4) and methanol (3:1) for 1 h. One milliliter of date seed extract-loaded nanoemulsion and suspension was placed into a separate preactivated dialysis bag (MW: 12,000–14,000 Da). Both the ends of dialysis bag were tied properly and suspended in a beaker filled with solvent system (20 mL). The whole setup was affined at 37°C ± 1°C with continuous stirring at 75 rpm for 24 h. At predetermined time intervals (0, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 h), drug samples (1 mL) were withdrawn and filtered over a 0.22-μm membrane filter. Withdrawn quantity of medium was replaced with an equal volume of fresh medium to achieve the sink condition. The analysis was done in triplicate. The withdrawn samples were analyzed by a UV spectrophotometer (UV-1800, Shimadzu) at 280 nm for the drug content. The release profile of the nanoemulsions were compared with the drug suspension.[19]

**Antioxidant activity**

The radical scavenging potential of methanolic extract of date seed in contrary to 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by analytical techniques UV spectrophotometry, at 517 nm. An aliquot (20, 40, 60, 80, 100, and 120 μg/mL) for methanolic extract of date seed was dissolved in different test tubes comprising 5 mL of methanol and 0.5 mL of 1 mM DPPH. α-Tocopherol (vitamin E) was used as the standard with the identical concentrations as that of test samples. A placebo solution with equal volume of methanol and DPPH was made, and this solution mixture was incubated at room temperature for half an hour.[20] The antioxidant activity was estimated using the following equation:

$$\% \text{ Scavenging activity} = \frac{\text{Ab} - \text{As}}{\text{Ab}} \times 100$$

where Ab denotes absorbance of blank and As denotes absorbance of sample.

All experiments were done in triplicate. The calculated IC₅₀ values are stated as mean ± SD.

**Result and Discussion**

**Extraction of components from date seed**

Methanol was used as a solvent for extraction of components using Soxhlet apparatus for 24 h. After 24 h, the solvent was evaporated under reduced pressure to obtain chocolate-colored solid mass and the percent yield was calculated to be 12% wt/wt [Figure 1].

**Solubility studies**

Solubility examinations were carried out to identify an appropriate oil phase for the effective formulation of date seed extract-loaded nanoemulsion to attain the drug in the solubilized form with optimal drug loading.
In the selected oils, the solubility of the date seed extract was observed maximum in Sefsol 218 (70 mg/mL) followed by IPM (32.48 mg/mL), triacetin (18.5 mg/mL), and oleic acid (20 mg/mL) [Figure 2]. Therefore, Sefsol 218 was chosen as oil phase for the formulation development. It has been reported that nanoemulsion comprising Sefsol 218 as oily phase provides a broader and stable nanoemulsion region when combined with a surfactant, Kolliphor RH40, and cosurfactant, PEG400.[21] Therefore, after performing a miscibility test of Sefsol 218-solubilized extract with Kolliphor RH40 and PEG400, it was observed that the selected surfactant and cosurfactant were completely miscible to each other.

**Ternary phase diagram study**

Ternary diagram is utilized to ascertain the presence of different zones of nanoemulsion. It graphically delineates the proportions of the three factors as positions in a symmetrical triangle. Typically, three distinct types of phages are observed during construction of a ternary phage diagram that is nanoemulsion state (transparent bluish nature), coarse emulsion (unstable, milky white) and liquid crystal (translucent gel like state). However, in this study, our interest was only in the nanoemulsion region and therefore only those points have been marked, which clearly showed the formation of nanoemulsion [Figure 3].

In this study, four different triangles were observed for the nanoemulsion region. F1 formulation contains Sefsol 218 as oil phase and Kolliphor RH40 as surfactant, and no cosurfactant was used in this case. It was found from the diagram that low quantity of oil might be solubilized with a higher concentration of surfactant. It generated a constricted range for nanoemulsion formation. Addition of a cosurfactant helps in improving the nanoemulsion region. An amphiphilic short-chain molecule is used for lowering the surface tension. Cosurfactants enter inside the surfactant monolayer, giving extra fluidity to the interfacial film and hence disturbing the liquid crystalline stages which are established when the surfactant film is excessively inflexible. This could be ascribed to the way that transient negative interfacial tension and liquid interfacial film are once in a while accomplished by the utilization of single surfactant, generally requiring the addition of a cosurfactant. The ternary plot of F2 formulation, which was composed of Sefsol 218 (oil phase), Kolliphor RH40 (surfactant), and PEG 400 (cosurfactant) with an $S_{\text{mix}}$ ratio of 1:1, demonstrated the development of a wider region of nanoemulsion. Transparent nanoemulsions with light bluish shade with the maximum solubilization of oil were achieved in this region. The formation of maximum nanoemulsions in this region may be accredited to lower interfacial tension and liquid interfacial film are once in a while accomplished by the utilization of single surfactant, generally requiring the addition of a cosurfactant. The ternary plot of F2 formulation, which was composed of Sefsol 218 (oil phase), Kolliphor RH40 (surfactant), and PEG 400 (cosurfactant) with an $S_{\text{mix}}$ ratio of 1:1, demonstrated the development of a wider region of nanoemulsion. Transparent nanoemulsions with light bluish shade with the maximum solubilization of oil were achieved in this region. The formation of maximum nanoemulsions in this region may be accredited to lower interfacial tension among oil and water phases, because maximum volumes of oil were solubilized with surfactant in this region. Furthermore, surfactant and cosurfactant blending in optimized amount causes massive oil phase solubilization. The availability of PEG 400 as cosurfactant diminishes the bending strain of interface and creates the interfacial film adequately adaptable to use up various arches obligatory to develop nanoemulsion over a wide scope.

![Figure 1: Methanolic extract of crown](image1)

![Figure 2: Quantitative solubility studies of date seed extract in different oils](image2)
of compositions. Observations from the ternary plot of F3 phase diagram showed higher nanoemulsion formation in the higher surfactant area; however, this section was relatively lower when compared with F2 diagram. Hence, lesser quantity of water was able to solubilize in this region; the nanoemulsion area was limited to boundaries adjacent to the surfactant area. This might be because of enhanced quantity of kolliphor RH40 in the formulation. Phase diagram achieved from F4 formulation ($S_{mix}$ ratio: 1:2) demonstrates

![Figure 3: Ternary phase diagrams of date seed extract nanoemulsions, F1 ($S_{mix}$ 1:0), F2 ($S_{mix}$ 1:1), F3 ($S_{mix}$ 2:1), and F4 ($S_{mix}$ 1:2)](image)

| Formulation code | $S_{mix}$ ratio | Percent oil used (%) | Percent $S_{mix}$ used (%) | Percent water used (%) | Thermodynamic stability test | Dispersibility | Result |
|------------------|-----------------|----------------------|-----------------------------|------------------------|----------------------------|----------------|--------|
|                 |                 |                      |                             |                        | Heating/cooling cycle      | Centrifugation | Freeze-thaw cycle | 0.1N HCl | Distilled water |
| F1               | 1:0             | 18.18                | 36.36                       | 45.45                  | X                          | –              | –      | –     | –     | Failed |
| F2               | 14.93           | 44.78                | 40.30                       | X                      | –                          | –              | –      | –     | –     | Failed |
| F3               | 10.00           | 30.00                | 60.00                       | ✓                      | ✓                          | ✓              | ✓      | ✓     | ✓     | Passed |
| F4               | 4.29            | 1.00                 | 90.00                       | ✓                      | ✓                          | ✓              | ✓      | ✓     | ✓     | Passed |
| F5               | 1:1             | 1.87                 | 13.12                       | 85.00                  | ✓                          | ✓              | ✓      | ✓     | ✓     | A B    | Passed |
| F6               | 3.00            | 23.00                | 74.00                       | ✓                      | ✓                          | ✓              | ✓      | ✓     | ✓     | B A    | Passed |
| F7               | 5.56            | 50.00                | 44.44                       | X                      | –                          | –              | –      | –     | –     | Failed |
| F8               | 2:1             | 5.80                 | 28.99                       | 65.22                  | ✓                          | ✓              | ✓      | ✓     | ✓     | B A    | Passed |
| F9               | 10.00           | 50.00                | 40.00                       | X                      | –                          | –              | –      | –     | –     | Failed |
| F10              | 14.93           | 74.63                | 10.45                       | X                      | –                          | –              | –      | –     | –     | Failed |
| F11              | 1:2             | 2.50                 | 17.50                       | 80                     | ✓                          | ✓              | ✓      | ✓     | ✓     | A A    | Passed |
| F12              | 5.00            | 45.00                | 50.00                       | ✓                      | ✓                          | ✓              | ✓      | ✓     | ✓     | A C    | Passed |
| F13              | 10.70           | 64.17                | 25.13                       | X                      | –                          | –              | –      | –     | –     | Failed |
| F14              | 13.70           | 41.10                | 45.21                       | ✓                      | ✓                          | ✓              | ✓      | ✓     | ✓     | A A    | Passed |

Table 2: Thermodynamic stability test of different formulation selected from phase diagrams

Table 3: Optimized formulation selected from phase diagram and thermodynamic stability study test at a difference of 3% vol/vol of oil

| Formulation code | Oil:$S_{mix}$ ratio | $S_{mix}$ ratio | % oil (vol/vol) | % $S_{mix}$ (vol/vol) | % Water (vol/vol) |
|------------------|---------------------|-----------------|-----------------|------------------------|-------------------|
| F3               | 1:3                 | 1:0             | 10              | 30                     | 60                |
| F6               | 1:9                 | 1:1             | 5.8             | 28.99                  | 65.22             |
| F8               | 1:5                 | 2:1             | 2.50            | 17.50                  | 80                |
| F11              | 1:8                 | 1:2             | 2.50            | 17.50                  | 80                |
the development of nanoemulsions in the watery-rich locale. It might be due to an enhanced amount of PEG 400 which causes a much reduced interfacial tension when compared with other systems.

**Thermodynamic stability tests**
All the developed nanoemulsions were evaluated for thermodynamic stability. Stable formulations were selected for preparation of drug-loaded nanoemulsion followed by further characterization. Stability test findings are reported in Table 2.

**Preparation of drug-loaded nanoemulsion**
Formulations that cleared all the stability tests and contained minimum amount of surfactant were selected for drug incorporation. Since it is assumed that a dose of drug is easily soluble in one mL of oil, therefore, drug was loaded in various oil concentrations which passed stability tests [Table 3].

**Physicochemical characterization of nanoemulsions**

**Visual appearance**
Physical observations were made for the developed formulations; clear and transparent nanoemulsions were found without any turbidity [Figure 4].

**Droplet size measurement and morphology**
Droplet size and size distribution were analyzed as shown in Figures 5 and 6. It was observed that all the formulations were in the nano size range with a low PDI value. The formulation F11 exhibited the lowest droplet size (23.14 ± 0.055 nm) with PDI, 0.166 ± 0.124 [Table 4]. The lower PDI value reflects the uniformity of nanoemulsions. The TEM image revealed that drug-loaded nanoemulsions were spherical in shape with the agreement of droplet size as noted by a zetasizer. Therefore, the outcomes were indicative of the fact that proper selection of oil, surfactant, and cosurfactant with definite concentrations are the crucial factors for achieving smaller droplets size with a stable nanoemulsion as shown in Figure 7.

**Drug content**
Drug content (date seed extract) in the optimized nanoemulsions was evaluated spectrophotometrically, which was in the range of 98.11%–99.61%. The drug content differed up to 1.0% between different formulations [Table 4].

**In vitro drug release**
Release studies of optimized formulations (F3, F6, F8, and F11) and conventional suspension of date seed extract were performed, and data revealed that release of drug from optimized formulations is tremendously
significant ($P < 0.001$) than release of drug from conventional suspension as depicted in Figure 8. Drugs released from the nanoemulsions were significantly higher as compared to that from drug suspension due to its nano-sized droplets, which offers a high surface area for solubilization, subsequently boosting solubility as well as dissolution rate. Comparative drug release data showed that the formulations F3, F6, F8, and F11 and drug suspension released 36.46% ± 1.82%, 39.43% ± 2.14%, 41.54% ± 1.78%, 57.51% ± 2.65%, and 26.44% ± 1.15% of cumulative drugs, respectively, at 24 h. It is probably due to the larger droplet size and viscosity of F3, F6, and F8, which could limit the drug release. On the basis of release studies, F11 formulation was selected for antioxidant activity.

**Antioxidant activity**

Antioxidant potential of date seed extract was determined by the DPPH assay in which the utilization of a stable free radical (DPPH) was assessed. Antioxidant activity was assessed based on the principle that when mixing the DPPH solution (used as an indicator) with an antioxidant, it donates hydrogen atom, which contributes to the reduced form “diphenylpicrylhydrazine” (non-radical). The color of the reaction mixture changed from purple to yellow and its absorbance was measured at a wavelength of 517 nm. The standard α-tocopherol compound was taken to compare the antioxidant activity of methanolic extracts of date seed. The IC$_{50}$ values of methanolic extract of date seed, nanoemulsion formulation, and α-tocopherol were 83.6 ± 1.78, 75.1 ± 1.09, and 98.1 ± 1.14 μg/mL, respectively.

### Table 4: Mean ($±$SD, $n = 3$) droplet size, polydispersity index, percent transmittance, refractive index, viscosity, conductivity, and drug content

| Formulation code | Droplet size (nm) | Polydispersity index | Percent transmittance | Refractive index | Viscosity (cP) | Conductivity (μS/cm) | Drug content (%) |
|------------------|------------------|----------------------|-----------------------|-----------------|---------------|---------------------|-----------------|
| F3               | 45.73 ± 0.208    | 0.363 ± 0.016        | 99.03 ± 0.0169        | 1.35 ± 0.015    | 17.14 ± 0.80  | 222.81 ± 1.90       | 98.41 ± 0.23    |
| F6               | 65.27 ± 0.092    | 0.449 ± 0.041        | 99.05 ± 0.35          | 1.34 ± 0.008    | 23.34 ± 0.30   | 316.22 ± 2.21       | 98.115 ± 0.42   |
| F8               | 32.47 ± 0.060    | 0.291 ± 0.210        | 99.06 ± 0.012         | 1.34 ± 0.011    | 20.20 ± 0.81   | 314.97 ± 2.85       | 99.15 ± 0.21    |
| F11              | 23.14 ± 0.055    | 0.166 ± 0.124        | 99.12 ± 0.0163        | 1.36 ± 0.046    | 12.30 ± 0.75   | 347.46 ± 1.10       | 99.61 ± 0.11    |

**Figure 7:** Transmission electron microscopy image of optimized nanoemulsion

**Figure 8:** In vitro release profile of date seed extract from different optimized nanoemulsion formulations (F3, F6, F8, and F11), and pure drug suspension (standard) in phosphate buffer, pH 5.6

**Figure 9:** Dose-dependent scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals by the methanolic extract of date seed and compared with standard drug α-tocopherol. Each value represents mean ± SD ($n = 3$). IC$_{50}$ values of methanolic extract of date seed, nanoemulsion formulation, and α-tocopherol were 83.6 ± 1.78, 75.1 ± 1.09, and 98.1 ± 1.14 μg/mL, respectively.
compounds and terpenes. The phytoconstituents existing in the extracts were verified by GC-MS, and it was found that extracts were rich in antioxidant principles, which are responsible for anti-inflammatory and anti-arthritis activity.[15]

**CONCLUSION**

In this research work, nanoemulsion containing date seed extract was successfully developed by utilizing Sefsol 218 as oil phase, kolliphor RH40 as surfactant, and PEG 400 as cosurfactant and further characterized for in vitro performance. The developed nanoemulsions were in nanometric size with a high drug content range (98.11%–99.61%) and were also thermodynamically stable. The in vitro release study showed significantly enhanced drug release when compared with drug suspension. Results from antioxidant activity indicate that optimized nanoemulsion has more antioxidant potential when compared with drug suspension. On the basis of our research findings, it could be concluded that the developed methanolic extract of date seed nanoemulsion was found to have more antioxidant potential and high drug release when compared with conventional suspension. The enhanced detailing could be a substitute for the treatment of RA. However, there is a need for further studies in terms of preclinical data to make it clinically reasonable.

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**Conflicts of interest**

There are no conflicts of interest.

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