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RESEARCH ARTICLE

Preparation of O/W nanoemulsion containg saffron aqueous solution and sesame oil and evaluation of its properties

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ABSTRACT

Objective(s): Oil-in-water (O/W) nanoemulsions are used in a wide range of biomedical applications due to the unique properties. Saffron is the valuable medicinal food product with many health benefits. Sesame oil is an edible vegetable oil with medicinal properties and potential applications. The preparation and evaluation of O/W nanoemulsion containing aqueous solution of saffron as hydrophilic medium and sesame oil as lipophilic medium were the purpose of this study.

Methods: O/W nanoemulsions were prepared by sesame oil, saffron aqueous solution and poly ethylene glycol (PEG) using span 80 surfactant and homogenizer at room temperature. Dynamic light scattering (DLS) and atomic force microscopy (AFM) were used to characterize sample size. Viscosity was applied to determine the rheological properties of the nanoemulsion at room temperature. The diameter of the inhibition zone was used to measure the antibacterial activity of samples against an important pathogen bacterium, Escherichia coli (E. coli). MTT assay was applied to evaluate the cytotoxicity of samples for HEK239 human cell line at different concentrations and time.

Results: Based on the results, the nanoemulsions showed the antibacterial activity with low toxicity. The presence of saffron and PEG increased the size, viscosity and antibacterial activity.

Conclusions: The study showed that nanoemulsions based on aqueous solution of saffron and sesame oil can be a good candidate for medicinal applications.

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INTRODUCTION

In recent years, nanotechnology has been used in various fields of science because of its great potential [1, 2]. Nanomaterials have become very widespread due to their small size and large surface area [3, 4]. Today, the widespread application of plant science in nanotechnology has increased [5, 6]. In recent years, the application of nanoemulsion has attracted much interest in various areas such as cosmetics, pharmaceutical compounds, food and agriculture industry [7, 8]. Nanoemulsions are classified between classical emulsions and microemulsions with a size range of 20 to 500 nm and generally include water-in-oil and oil-in-water [9, 10]. The O/W nanoemulsions can contain a lipophilic bioactive ingredient as an oil phase and an aqueous medium [11, 12]. Nanoemulsion can be prepared by homogenization method as the high energy technique in the presence of emulsifier [13]. Recently, the herbal nanoemulsions have been developed for medical applications [14, 15].

Newly, the use of saffron has expanded despite its high price due to the importance of using natural products and medicinal food with therapeutic properties and anti-bacteria

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Table 1. The percentage of nanoemulsion component.

Sample	Sesame oil (mL)	Span 80 surfactant (mL)	Saffron (g)	PEG (g)
I	4	2	-	-
II	4	2	0.25	-
III	4	2	-	2
IV	4	2	0.25	2

activity [16]. Also, sesame oil is used as an herbal medicine in the tropics [17, 18]. The antimicrobial activity of sesame has become an interesting and expanding application [19]. The high prevalence of bacterial infections and the increasing resistance of bacteria expand the need for new antibiotics [20]. Nanotechnology is increasingly using nanomaterials and nanostructures for antibacterial applications and is considered as an approach to eliminate or reduce the activity of microorganisms [21, 22]. Medicinal plants can be used as natural antibacterial agents to increase efficacy and inhibit bacterial growth [23, 24]. Essential oil as one of the compounds derived from the plant can have antimicrobial activity. The antimicrobial activity of this natural substance has been greatly increased, mainly in response to consumer concerns about the safety of synthetic oral additives [25]. Recently, the use of essential oils in nanoemulsions has had a special place [26-28]. These volatile substances can cause therapeutic and pharmacological properties [29, 30]. In the present study, nanoemulsion was prepared containing sesame oil, saffron aqueous solution and PEG and evaluated antibacterial activity against *E. coli* Gram-negative bacterial and cytotoxicity on HEK239 human cell line.

MATERIALS AND METHODS

Materials

All chemicals used had analytical grade. Ultra-pure water was used to prepare all reactant solutions. Span 80 and PEG 1000 were purchased from Merck Millipore (Darmstadt, Germany). Sesame essential oil and saffron bought from Adonis Gol Daro (Iran).

Characterization

The size and size distribution of nanoparticles were evaluated by dynamic light scattering (ZEN314, England). The morphology and size were investigated by atomic force microscopy (Nanosurf, Switzerland). The viscosity was studied by rheological properties based on spindle velocity for nanoemulsions using Reologica InstrumentsAB (Lund, Sweden). Disc diffusion method was used to evaluate the antibacterial activities of samples against *Escherichia coli* bacteria, ATCC 1399, that procured from Islamic Azad University. MTT assay was applied to investigate the cytotoxicity of nanoemulsions for HEK239 human cell line at three different concentrations for 1 and 5 days.

Nanoemulsion preparation

The optimal nanoemulsion was prepared from a mixture of 4 mL sesame oil, 0.25 g saffron in aqueous solution and 2 mL span 80 surfactants by homogenizing at 15000 rpm for 15 min in presence or the absence of 2 g PEG (Table. 1).

RESULTS AND DISCUSSION

DLS

Dynamic light scattering was used to determine the mean particle diameter and the particle size distribution of the nanoemulsions. Based on the results, a sharp peak with a narrow distribution was observed.

Based on these results, the size was observed for the nanoemulsions including 62 nm (I), 71 nm (II), 75 nm (III) and 134 nm (IV) and the presence of saffron and PEG was caused to the increase of the size. The DLS results are based to previous report [7].

AFM

Fig. 2 was shown the AFM images for the nanoemulsions with spherical morphology. The AFM result approved a narrow distribution of the DLS results. AFM results are according to previous research [31].

Rheological properties

The viscosity was investigated for nanoemulsions (Table. 2). In the same spindle speed, the viscosity of nanoemulsions was as follows:

nanoemulsions (I) < nanoemulsions (II) = nanoemulsions (III) < nanoemulsions (IV).

The presence of saffron and PEG increased the viscosity of nanoemulsions. According to previous

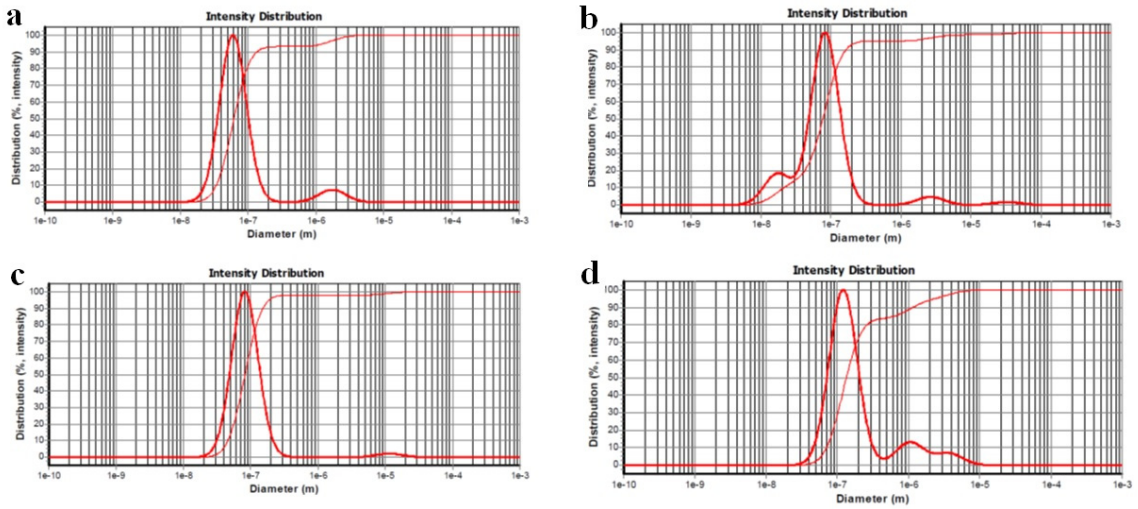

Fig. 1. DLS diagram of nanoemulsions a) (I), b) (II), c) (III) and d) (IV).

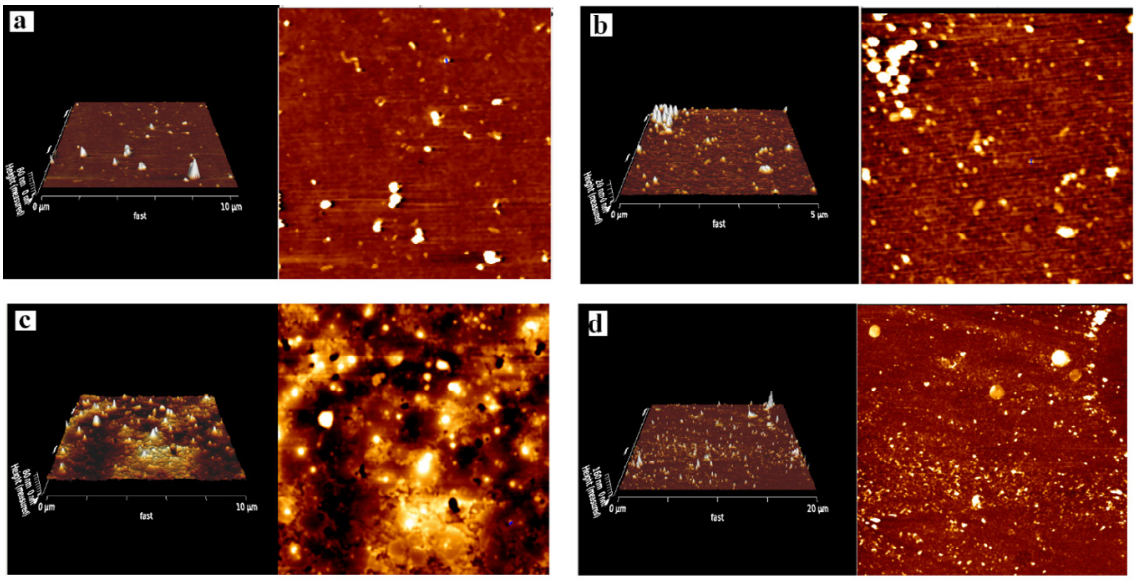

Fig. 2. AFM images of nanoemulsions a) (I), b) (II), c) (III) and d) (IV).

Table 2. Rheological properties of nanoemulsions.

Sample	Spindle speed (round per minute)	Viscosity (centipoise)
I	60	3
II	60	4
III	60	4
IV	60	5

studies, the presence of the polymer increases the rheological properties [7, 32]. This work is the first report on the increase of rheological properties by saffron.

Antibacterial activity

The antibacterial activity was investigated by determination of inhibition zone diameter of samples against a Gram-negative bacterial. The

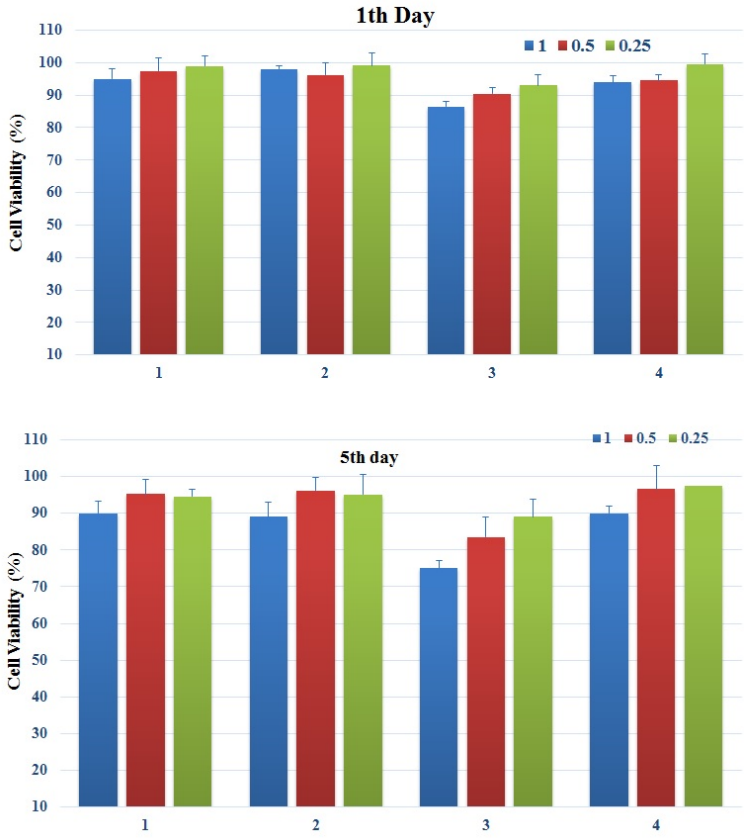

Fig. 3. The curves of nanoemulsion cytotoxicity in different times and concentrations for 1) (I), 2) (II), 3) (III) and 4) (IV).

Escherichia coli cells were cultivated on Mueller-Hinton Agar and incubated at 37 °C for 1 day. The inhibition zone diameter of nanoemulsion was 2 mm (I), 5 mm (II), 8 mm (III) and 9 mm (IV). According to the result, saffron and PEG increased the antibacterial activity of nanoemulsion. The antibacterial activity is reported in the presence of nanoemulsion [7], PEG [33] and saffron [34].

MTT assay

MTT assay was used to investigate the cytotoxicity of nanoemulsion for HEK239 human cell line at 0.25, 0.5, and 1% (v/v) concentrations for 1 and 5 days. Based on the result, the two effective factors in the cytotoxicity of nanoemulsions were concentration and time. Increasing concentration and time increased cytotoxicity due to decreased cell viability. Based on the analysis of the results, on the fifth day, the rate of cell death increased in samples 3, and this increase in death is somewhat significant. The advantages of the nanoemulsion include acceptable toxicity over a longer period of time compared to a previous report by researchers [7].

CONCLUSIONS

The present study showed that the nanoemulsion with sesame oil and saffron aqueous solution in the presence of PEG could be a good candidate for antibacterial activity against *Escherichia coli* with low cytotoxicity for HEK239 human cell. The presence of saffron and PEG leads to increased size, viscosity and antibacterial activity. Nanoemulsion can be a cost-effective method for future use in food and pharmaceutical applications.

COMPETING INTERESTS

The author declares there are no conflicts of notice in preparing the manuscript.

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