ABSTRACT

Objective: The purpose of this study was to develop a nanoemulgel containing vegetable oil of carrot seed oil as an effective natural sunscreen and skin anti-aging.

Methods: Nanoemulgels containing 4% carrot seed oil were formulated in three formulas with different ratios of Tween 80 and Sorbitol and prepared by using the high-energy emulsification method. The nanoemulgels were determined for the organoleptic characteristic, globule size, pH, physical stability during storage for 12 w at three different temperatures (room, high and low temperature), centrifugation, and cycling test. The Sun Protection Factor (SPF) value was determined by UV spectrophotometric method and the effectiveness of anti-aging was evaluated by using a skin analyzer and the results were compared with sunscreen emulgel.

Results: Nanoemulgel containing 4% carrot seed oil with a ratio of Tween 80 as surfactant and Sorbitol as cosurfactant 40 and 20 resulted in the smallest mean droplet size of 338.34 nm and the sizes were increased during 12 w of storage at room temperature but still in the nano size and this nanoemulgel did not show phase separation or still stable. These nanoemulgels were also stable after the centrifugation and cycling test. The emulgel preparation was not stable or showed phase separation after the centrifugation test. The SPF value obtained from the nanoemulgel was 2.08±0.22 and these values were higher than the sunscreen emulgel (1.94±0.27). The pore size, spot, and wrinkles of the volunteer skin were reduced after using the nanoemulgel containing 4% carrot seed.

Conclusion: The sunscreen and skin anti-aging activity of nanoemulgel preparation containing 4% carrot seed oil with a ratio of surfactant Tween 80 and co-surfactant Sorbitol 40 and 20 were more effective compare with emulgel preparation.

Keywords: Carrot seed oil, Nanoemulgel, Sunscreen, Skin anti-aging

INTRODUCTION

UV radiation in the skin induces oxidative free radicals, such as superoxide, hydrogen peroxide, and highly reactive hydroxyl radicals that strongly attack macromolecules such as proteins, lipids, RNA, and DNA, changing their structure and interfering with their function, which can cause oxidative stress [1]. Oxidative stress can cause cell damage and death in the skin and cause skin aging. The visible signs of aging on the skin, including dry skin appearance, scalping, wrinkling, damage and death in the skin and cause skin aging. The visible signs of aging on the skin, including dry skin appearance, scalping, wrinkling, and co-surfactant Sorbitol 40 and 20 were more effective compared with emulgel preparation. The sunscreen and skin anti-aging activity of nanoemulgel preparation containing 4% carrot seed oil with a ratio of surfactant Tween 80 and co-surfactant Sorbitol 40 and 20 were more effective compare with emulgel preparation.

Nanoemulgels were prepared by mixing the obtained nanoemulsion into a gel matrix. The nanoemulgel has the advantage that it can increase the stability of the nanoemulsion by reducing surface tension, improve the patient’s acceptability because it had good dispersibility, was not greasy, easy to apply, and increase the penetration of lipophilic active ingredients like vegetable oil (carrot seed oil) into the skin thereby increasing the effectiveness of the active ingredients [11, 12].

MATERIALS AND METHODS

The active ingredient used in this study was Carrot Seed Oil (Happy green store, Jakarta Indonesia), Carbopel 940, sodium carboxymethyl cellulose, glycerol, propylene glycol, sorbitol, methylparaben, propylparaben, Tween 80, Triethanolamine, Span 80, buffer solution pH 7.01, buffer solution pH 4.01 were purchased from CV Rudang Jaya, Medan, Indonesia. All ingredients were analytical grade.

Nanoemulsions containing carrot seed oil were prepared through a high-energy emulsification method [13, 14] by using a magnetic stirrer and ultrasonicator. Tween 80 and sorbitol are used as surfactants and co-surfactants. The nanoemulsion system consists of the oil and the water phase. The oil phase consists of carrot seed oil and sorbitol. In the water phase, methylparaben and propylparaben as preservatives were dissolved in distilled water, then heated by using the hot plate (Fisons); this solution was added Tween 80 and stirred at 3000 rpm by magnetic stirrer (Boeco, Germany) for 8 h. The oil phase was mixed into the water phase and stirred at 3000 rpm with a magnetic stirrer (Boeco, Germany) for 8 h, then sonicated for 30 s by ultrasonicator (Ultrasonic Cleaner Branson 1510 E-MT, USA) until a transparent nanoemulsion was formed. Carbopel 940 was dispersed in pure water and added with TEA to pH-6.5 to obtain a gel base of 1% Carbopel 940 solution [15]. Nanoemulgel were prepared by mixing the obtained nanoemulsion containing carrot seed oil with a gel base of 1% Carbopel 940 (ratio
of nanoemulgels and gel base 4:1), then stirred using a magnetic stirrer at 3000 rpm for 10 h and ultrasonicated for 2 h until a transparent nanoemulgels were produced [16].

The preparation of emulgel containing 4% carrot seed oil was prepared by mixing carrot seed oil and Span 80, then this oil phase was heated to 70 °C. The preservatives (methyl and propyl parabens) were dissolved in propylene glycol and glycerol and added CMC Na solution and this water phase was heated to 70 °C. After that, the oil phase was added to the water phase and stir with a magnetic stirrer for 45 min to produce a homogenous emulsion [17]. Emulgel was obtained by mixing the obtained emulsion with a gel base of 2% of Carbopol 940 (ratio of emulsion and gel 1:4) with gentle stirring for 10 min.

The mean droplet size for nanoemulgels and emulgel formulations was measured by Particle Size Analyzer Fritsch Analysette 22 NanoTec. The pH measurement is done using 1% nanoemulgol solution in pure water with a digital pH meter (Hanna instrument). Viscosity measurement was carried out using the NDJ-8S Viscometer and viscosity value was measured every 2 w for 12 w of storage at room temperature. Physical stability evaluation was done by storing it is in the freezer at 4±2 °C for 24 h and repeated in 6 cycles. Centrifugation test was done using centrifuges (Hitachi CF 16 R X II, Japan) with a rotation of 3750 rpm for 5 h at 25±2 °C. The physical stability of the nanoemulgels and emulgel was observed [19].

Anti-aging efficacy measurement was performed on 6 volunteers by using a skin analyzer (Aram, Huvis, Co., Ltd, Korea). The volunteers were accommodated in an air-conditioned room at 25±1 °C and 45±5% relative humidity for 15 min before the measurements. The selected nanoemulgel preparation containing 4% carrot seed oil was applied 2 times every day for 28 d. The moisture content, pore, evenness, spot, and wrinkles of skin were measured before the application of nanoemulgel on day 0 and after application at days 7, 14, 21, and 28. Approval to conduct the in vivo studies was obtained from the Research Ethics Committee of Universitas Sumatera Utara (No. 166/KEP/USU/2020).

RESULTS

Carrot seed oil nanoemulgol was prepared by using a variation of the ratio between Tween 80 as surfactant and sorbitol as co-surfactant. The resulting nanoemulgol was added with a gel solution of carbopol 940 to produce nanoemulgel. The composition of nanoemulgel and emulgel can be seen in tables 1 and 2.

| Table 1: Composition of carrot seed oil nanoemulgels |
|-----------------------------------------------------|
| **Quantity of 100 ml (%w/v)** | **Ratio of nanoemulgol with gel base** |
|-------------------------------|-------------------------------------|
| Ingredients of nanoemulgol     | F1   | F2   | F3   |
| carrot seed oil                | 5    | 5    | 5    |
| Tween 80                       | 40   | 38   | 36   |
| Sorbitol                       | 20   | 22   | 24   |
| Methylparaben                  | 0.10 | 0.10 | 0.10 |
| Propylparaben                  | 0.02 | 0.02 | 0.02 |
| Distilled water to             | 100  | 100  | 100  |
| Ingredients of gel base        |      |      |      |
| Carbopol940                    | 1.0  | 1.0  | 1.0  |
| TEA                            | 1.0  | 1.0  | 1.0  |
| Distilled water to             | 100  | 100  | 100  |

| Table 2: Composition of carrot seed oil emulgel |
|------------------------------------------------|
| **Quantity of 100 ml (%w/v)** | **Ratio of nanoemulgel with gel base** |
|-------------------------------|-------------------------------------|
| Ingredients of emulsion carrot seed oil | 5.00 | 1.26 |
| Tween 80                      | 1.00 | 0.02 |
| Span 80                       | 3.73 | 0.02 |
| CMC Na                        | 1.00 | 80 ml|
| Propylene glycol              | 1.00 | 80 ml|
| Methylparaben                 | 0.10 | 20 ml|
| Propylparaben                 | 0.02 | 20 ml|
| Distilled water to             | 100  |      |
| Ingredients of gel base        |      |      |
| Carbopol940                   | 1.00 |      |
| TEA                            | 1.00 |      |
| Distilled water to             | 100  |      |

All preparations of carrot seed oil nanoemulgels were yellow and translucent and this appearance did not change during 12 w of storage at room temperature but the consistency of carrot seed oil emulgel becomes liquid as shown in fig. 1.

The nanoemulgel containing carrot seed oil obtained in this study had a smaller droplet size than the emulgel formulation. The emulgel preparation has a droplet size of 5028.37 nm. The droplet size of the nanoemulgel containing carrot seed oil increased during 12 w of storage at room temperature, but the droplet was still in the nanosize as presented in table 3.

Table 4 shows that the decrease in pH and viscosity value of the emulgel containing carrot seed oil was greater than that of the nanoemulgel preparations during 12 w of storage at room temperature.
Fig. 1: Appearance of carrot seed oil nanoemulgels (F1, F2, and F3) and emulgel (E)

Table 3: The mean droplet size for nanoemulgel preparations

| Formula code | Time (w) | Mean droplet size (nm) |
|--------------|----------|------------------------|
|              | 0        | 338.34                 |
| F1           | 4        | 411.86                 |
|              | 8        | 512.27                 |
|              | 12       | 607.23                 |
| F2           | 0        | 401.29                 |
|              | 4        | 416.27                 |
|              | 8        | 532.15                 |
|              | 12       | 645.20                 |
| F3           | 0        | 401.29                 |
|              | 4        | 470.31                 |
|              | 8        | 583.63                 |
|              | 12       | 688.15                 |

Table 4: Viscosity and pH value of nanoemulgel and emulgel containing carrot seed oil

| Formula code | Time (w) | pH (n=3)     | Viscosity (mPas) (n=3) |
|--------------|----------|--------------|------------------------|
| F1           | 0        | 6.90±0.00    | 499.00±0.00             |
|              | 4        | 6.80±0.00    | 454.33±1.15             |
|              | 8        | 6.53±0.06    | 439.00±1.73             |
|              | 12       | 6.13±0.06    | 422.33±2.52             |
| F2           | 0        | 6.87±0.06    | 486.33±1.15             |
|              | 4        | 6.70±0.00    | 455.00±0.00             |
|              | 8        | 6.47±0.06    | 441.33±1.15             |
|              | 12       | 6.13±0.06    | 417.33±2.31             |
| F3           | 0        | 6.87±0.06    | 475.33±3.51             |
|              | 4        | 6.77±0.06    | 454.00±1.73             |
|              | 8        | 6.43±0.06    | 437.67±1.15             |
|              | 12       | 6.07±0.06    | 411.67±2.89             |
| Emulgel      | 0        | 6.97±0.06    | 1998.00±0.00             |
|              | 4        | 6.67±0.06    | 1530.00±4.62             |
|              | 8        | 6.37±0.06    | 1118.33±2.89             |
|              | 12       | 5.87±0.06    | 946.67±2.89             |

The color and odor of nanoemulgel remain unchanged; there is no phase separation after storage for 12 w at low and high temperatures as can be seen in fig. 2.

Fig. 2: Appearance of carrot seed oil nanoemulgels after storage for 12 w at low temperature (A) and high temperature (B)

The result of the cycling test of nanoemulgel preparation did not find any phase separation (fig 3). There was phase separation in the emulgel preparation after the centrifugation test, but the physical form of nanoemulgel preparations did not change as shown in fig. 4.
To study the morphology and distribution of nanoemulgel and emulgel, transmission electron microscopy photomicrographs were taken, as shown in fig. 5. It was observed that nanoemulgel has a uniform dispersion, spherical without any aggregation and the droplet size was smaller than emulgel preparation.

The nanoemulgel containing carrot seed oil showed a higher SPF value than emulgel preparation (table 5). The results of SPF determination revealed that carrot seed oil in the form nanoemulgel exhibits greater UVR protection.

Table 5: SPF value of nanoemulgel and emulgel containing carrot seed oil

| Formulation code | Sun protection factor (SPF) value |
|------------------|----------------------------------|
| F1               | 20.2±0.218                       |
| F2               | 19.3±0.280                       |
| F3               | 16.5±0.190                       |
| Emulgel          | 13.9±0.266                       |

Notes: Data is presented as mean±SD, n=6

Nanoemulgel F1 which has the highest SPF values were chosen to evaluate the effectiveness of anti-aging on skin volunteers. The parameters of anti-aging activity evaluation include measurement of moisture content, pore size, evenness, spot, and wrinkles. The skin anti-aging measurement results can be seen in table 6 and table 7. The moisture content of volunteers increases after the application of nanoemulgel and emulgel from dehydrated skin to normal skin. The pore size of the volunteer skin was reduced from
some large (41.3±2.8) to excellence (18.0±1.4), the spot from many spots (40.5±2.7) to little spot (18.6±3.0), and wrinkles from wrinkles (43.3±3.5) to approach fine line (20.5±2.1) after using the nanoemulgel containing 4% carrot seed oil (F1) for 4 w. The volunteer’s skin becomes smooth (27.1±1.4) after using nanoemulgel for 2 w. The condition of pore size, spot, evenness, and wrinkles of volunteer skin did not change after using emulgel for 4 w.

Table 6: Moisture content (%) results of nanoemulgel (F1) and emulgel containing carrot seed oil 4% on skin volunteers

| Formulation code | Time (Week) | 0      | 1      | 2      | 3      | 4      |
|------------------|------------|--------|--------|--------|--------|--------|
| F1               |            | 27.8±1.4 | 31.5±1.3 | 35.3±1.2 | 38.6±1.2 | 42.1±0.7 |
| Emulgel          |            | 28.1±0.4 | 29.8±1.4 | 32.1±1.1 | 34.0±1.2 | 35.8±1.1 |

Notes: Data is presented as mean±SD, n=6, Dehidrated 0-29%, Normal 30-50, Hidrated 51-100

Table 7: Pore, evenness, spot, wrinkles measurement results on skin volunteers

| Formulation code | Time (week) | Average of score | Pore       | Evenness   | Spot       | Wrinkles  |
|------------------|-------------|------------------|------------|------------|------------|-----------|
| Nanoemulgel(F1)  | 0           | 41.3±2.8         | 36.3±1.6   | 40.5±2.7   | 43.3±3.5   |
|                  | 1           | 35.5±2.1         | 33.5±3.3   | 34.8±2.8   | 37.8±3.0   |
|                  | 2           | 28.3±1.4         | 31.1±2.2   | 29.0±3.0   | 30.6±3.0   |
|                  | 3           | 24.0±1.4         | 29.3±1.6   | 23.8±3.3   | 23.8±2.5   |
|                  | 4           | 18.0±1.4         | 27.1±1.4   | 18.6±3.0   | 20.5±2.1   |
| Emulgel          | 0           | 40.0±2.0         | 38.0±1.5   | 41.0±2.3   | 41.6±2.1   |
|                  | 1           | 37.6±2.0         | 36.3±1.7   | 36.3±2.3   | 38.5±1.6   |
|                  | 2           | 35.6±1.9         | 34.3±0.8   | 34.1±1.7   | 33.6±3.3   |
|                  | 3           | 33.6±1.7         | 32.8±0.7   | 32.1±1.7   | 30.6±3.3   |
|                  | 4           | 31.6±1.8         | 31.1±0.7   | 29.8±1.7   | 27.1±2.2   |

Notes: Data is presented as mean±SD, n=6

DISCUSSION

There are two methods for nanoemulsion preparation: the high-energy method in which a mechanical device is used and the low-energy method in which the chemical potential of the component is used [21]. In this study nanoemulgel containing carrot seed oil preparations have been successfully formulated using Tween 80 as a surfactant, Sorbitol as a co-surfactant, and Carbopol 940 as a gelling agent used in addition to the formulation to facilitate the stabilization process. Carbopol 940 has advantages such as being non-toxic, biocompatible, bioadhesive (long residence time on the skin) [22]. The amount of Carbopol 940 used is 0.5%, which was sufficient to produce nanoemulgels translucent, non-greasy, and easy to spread. The addition of Trietanolamin in the formulation can form a good and stable gel matrix at a pH around 6.7. The pH value of nanoemulgels obtained still meets the pH standard for topical preparations suitable with skin pH balance (4.5-6.5) [23] and the pH value and activity skin anti-aging of nanoemulgels are more effective compare with emulgel preparation. The emulgel preparation of 20.28±0.22 and most effective of activity skin anti-aging. The SPF value of nanemulgel preparations is higher than that of emulgel, so it will absorb more ultraviolet light, which results in higher SPF values.

Oxidative stress is considered to be one of the main mechanisms involved in skin aging [25]. Carrot seed oil contains beta carotene that has antioxidant activity can be useful for preventing and treating skin aging. However, beta carotene is very lipophilic, so that penetration into deep layers of the skin is only slightly; therefore in this study, the carrot seed oil was formulated in nanoemulgel to increase the penetration of antioxidants for increasing anti-aging activity.

The anti-aging activity of nanoemulgel containing 4% carrot seed oil (F1) was higher than that of emulgel preparation with the same concentration of carrot seed oil. This is because the smaller globule size of nanoemulgel can pass through the pores on the skin easily, thereby increasing the penetration of the active ingredients into the skin [26, 27].

CONCLUSION

Carrot seed oil can be formulated into nanoemulgels using Tween 80 as surfactant and Sorbitol as co-surfactant and physically stable on stability test. Nanoemulgel containing 4% carrot seed oil with a ratio of Tween 80 and Sorbitol 40 and 20 (formulation code F1) has the smallest droplet size of 33.8±1.4 nm and showed the highest SPF value of 20.8±0.22 and most effective of activity skin anti-aging. The SPF value and activity skin anti-aging of nanoemulgels are more effective compare with emulgel preparation. The emulgel preparation showed phase separation after the centrifugation test.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors report no conflicts of interest in this work.
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