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Preparation and evaluation of clove oil in emu oil self-emulsion for hair conditioning and hair loss prevention

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ABSTRACT

Introduction: From a consumer perspective, developing a hair care formulation that offers multi-purpose products to enhance routine hair care such as conditioning, cleaning and grooming hair and stimulating hair follicles is important. Eugenol comprising about 70% of clove essential oil shows an androgenic activity and stimulates hair root to feed and hence could be a good candidate for developing an anti-hair loss formulation. Thus in hair research, hair follicle is of great interest. The aim of this study was to develop a self-emulsifying product containing eugenol in emu oil as a carrier.

Methods: Eugenol was identified in clove oil extraction by UV spectrophotometer. Emu oil was characterized according to national oil standards. All formulations were prepared and best one was selected for further pharmaceutical examinations such as pH, particle size, content uniformity and drug release. The optimum formulation was clinically evaluated on rats back compared with minoxidil standard lotion as a positive control and distilled water as a negative control.

Results: The selected formulation was demonstrated to condition hair with grooming and enhanced hair growth with longer lag time compared with minoxidil but after one week the hair growth accelerated.

Conclusion: The formulation containing clove oil in emu oil self-emulsion shows a conditioning and grooming property with hair shaft repair and hair growth.

Implication for health policy/practice/research/medical education:
Eugenol comprising about 70% of clove essential oil exerts an androgenic and antibacterial activities and stimulates hair root to feed and hence could be a used by humans for these purposes.

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Introduction

Hair is one of the vital fragments of the body derived from ectoderm of skin and contains a protein called keratin that is produced in hair follicles in the outer layer of skin (1-3). Hair loss or alopecia is one of the most common problems of many communities causing many economical and physiological problems (4). Alopecia is a dermatological disorder that has been known for more than 2000 years and is considered a common problem in cosmetics as well as primary health care practice (1,2). Normally, 50-100 hairs are lost per day and an increase in lost hairs up to more than 100 is considered hair loss (3-5). There are many types of hair loss and the most common type is referred to as male-pattern baldness (when it occurs in women it is called female-pattern baldness) which affects over 95% of people with hair loss (6,7). Androgenetic alopecia is hereditary thinning of the hair induced by androgens in genetically predisposed men and women (1,2,8). Alopecia areata is an autoimmune illness affecting nearly 2% of the US population. Alopecia areata affects both sexes similarly and occurs at all ages, although children and young adults are affected most often (6-8). The treatment of hair loss is sometimes difficult because of deficient efficacy and limited options (4). Two drugs have been approved by the Food and Drug Administration (FDA) for the treatment of androgenetic alopecia, minoxidil and finasteride. Minoxidil is a special lotion applied twice a day to enhance blood supply to the follicles and papillae and encourage hair growth (6,7). Minoxidil is a powerful vasodilator and appears safe for

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long-term treatment and anagen phase of hair growth, and enlarges miniaturized and suboptimal follicles (8-11). The main adverse reactions are itching, contact dermatitis and dryness (10,12). Finasteride (Propecia)®, taken once a day, is a an oral medication that specifically decreases the production of dihydrotestosterone by blocking the enzyme vital to its formation. Treatments of alopecia areata include steroids (injection or topical ointment), minoxidil and anthralin cream. Corticosteroid may be injected into the bald patches or applied directly to the skin as a lotion to stimulate hair growth. Usually, hair begins to grow again within some weeks, and the injections are repeated within a month. Anthralin cream may also be applied to the hairless area; this irritant is used every day and rinsed off an hour later. The treatment usually encourages hair growth within two to three months (6,7).

The purpose of this study is to prepare self-emulsion using appropriate amounts of eugenol and emu oil as an emollient agent to reduce skin irritation and increase penetration.

The emu oil can simply penetrate into skin because of containing large amounts of oleic acid and similarity to human sebum. It is an excellent transdermal carrier which penetrates into the skin, increases the potency of topical medications such as eugenol and provides long-lasting effectiveness. Emu oil helps to repair scar tissue. It also accelerates the development of fresh skin cells by delivering the required bio-nutrients deep into skin where fresh cells form and decrease the buildup of wound tissue (13,14).

Materials and methods

Clove essential oil (eugenol) was supplied from Golchai Co. (Iran), cetrimonium chloride and coconut fatty acid diethanolamin were supplied from Merck Co. (Germany) and polysorbate 80 from (Croda Chemicals Ltd, UK), Emu oil was obtained from Abyaneh Cosmetic Company (Isfahan, Iran). All other ingredients used in this study were of analytical grade.

Authentication of eugenol

Eugenol was authenticated according to USP and BP pharmacopeia by UV spectroscopy.

Preparation of standard curve

In order to generate standard curve, 19 mg of eugenol was accurately weighed and solubilized in 19 ml ethanol 96%. This solution was diluted to obtain standard solutions at the concentrations of of 5-50 µg/mL. The absorbance of standard solutions was measured by UV/Visible spectrophotometer (Shimadzu, UV mini-1240CE) at 282 nm and the standard curve was generated (Figure 1).

Preparation of self-emulsion

To develop a stable emulsion, the formulations were developed with different amounts of emulsifiers with experimental design. Nine experiments were suggested by software according to input variable and expected output specification. The compositions of prepared formulations are shown in Table 1. The oil phase of

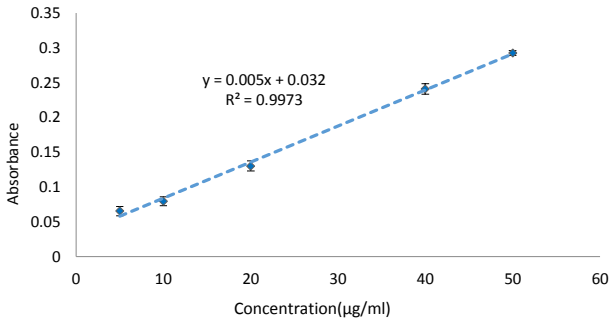

Figure 1. Ultraviolet spectrum of standard eugenol.

emulsion was prepared by mixing emu oil and eugenol 1%. The oil phase was separately heated to 40-50°C to achieve homogeneity. Then the aqueous phase was added to the oily phase with continuous stirring at 800 rpm to obtain a clear, transparent micro emulsion. Based on the primary evaluation of organoleptic and centrifuge test of the prepared emulsions, formulation 6 (F6) was selected to finish evaluation.

Evaluation of the selected formulation

The following physicochemical parameters were used for the evaluation of formulation:

Determination of particle size

The particle size of emulsion was determined by zeta analyzer (Malvern Instruments Ltd.).

Organoleptic evaluation

The prepared self-emulsion was examined visually for color and homogeneity.

Centrifuge test

The prepared formulation was centrifuged at 3000 rpm for 30 minutes (HETTIC D-7200, Germany) 24 hours after preparation and then once a week for 28 days (15).

Determination of pH

pH of the prepared formulation was measured by a digital pH meter (Metrohm, Switzerland) (15). The determinations were carried out in triplicate and the average value of three readings was recorded.

Freeze-thaw cycle

Freeze-thaw treatment of the emulsion was performed closely after preparation. Samples (20 mL) were stored at -20°C for 48 hours. The frozen samples were subsequently thawed at room temperature for 48 hours (16). This test carried out in triplicate for each sample.

Drug content

To determine drug content, a certain amount of emulsion (10 puffs equal to 1 g of emulsion) was collected and introduced into a screw-capped tube. Then phosphate buffer (pH 7.4) was added to the emulsion up to the volume of 50 mL. After shaking for 2 hours in orbital water bath shaker (Gallen KAMP, Germany) at 37°C, the diluted emulsion was filtered through a 0.45 µm Whatman filter. The amount of eugenol was determined spectrophotometrically (Shimadzu, UV mini-1240CE) by measuring the absorbance of the filtrate at 282 nm (17).

Table 1. Self-emulsion formulation (oil/water%)

	Formulation								
Ingredient (g)	1	2	3	4	5	6	7	8	9
Eugenol	1	1	1	1	1	1	1	1	1
Emu oil	5	5	5	5	5	5	5	5	5
Cetrimonium chloride	0.5	2	2	1	1	0.5	2	1	0.5
Cremophor	6	2	6	2	6	2	4	4	4
Cetyl Alcohol	4	4	3	3	2	2	2	4	3
Tween 80	5	3	1	5	3	1	5	1	3
Coconut fatty acid diethanolamin	2	2	2	2	2	2	2	2	2
Canola oil up to 100 ml	76.5	81	80	81	80	86.5	79	82	81.5

This procedure was performed for an emulsion system containing no drug as the blank.

Stability test

The stability was examined at 8°C (in refrigerator), 25°C (in room) and 40°C (in oven) (15,18). At 1 week intervals for 1 month, drug content and physical appearance (organoleptic characteristics) were examined.

In-vitro drug release study

Franz diffusion cell (25 mL volume) was used to study the drug release. The receiver compartment was filled with ethanol 96% obtained after examination of sink condition and 10 puffs (equal to 1 g emulsion) of self-emulsion were applied on the surface of cellulose acetate membrane. The membrane was clamped between the donor and the receiver compartments. The donor compartment was exposed to the receiver compartment at 37°C temperature. The solution on the receiver compartment was stirred by magnetic stirrer. At predetermined time intervals, 0.5 mL of solution from receiver compartment was pipetted out and closely replaced with fresh 0.5 mL of receiver medium. After appropriate dilution the drug concentration on the receiver medium was determined spectrophotometerically at 282 nm. The experiment was carried out in triplicate. To study drug release kinetics, data obtained from in vitro investigation of release were fitted to the zero, first and Higuchi kinetic models (16,19,20).

Kinetic analysis of drug release

To study drug release kinetics, data obtained from in vitro investigation of release were fitted in Zero, First and Higuchi kinetic models (20-22).

Animal study

Male Wistar albino rats weighing 200-250 g were used for in vivo studies. They were kept in standard environmental conditions with free access to special diet and drinking water. All animal experiments were carried out in accordance with the guidelines of Institutional Animal Ethics Committee of Isfahan University of Medical Sciences.

Skin irritation test

Three healthy male rats were selected for this study. Each rat was separately kept in a cage during the study period. The hair from the back of each rat (test sites of 1 cm^2) was shaved on the side of the spine to expose sufficiently large test areas. The test sites were cleaned with surgical spirit and 1 g of F6 was applied over the respective test sites of one side of the spine. The test sites were observed for erythema and edema for 48 hours after application (23,24).

Application of test formulations for hair growth evaluation

The rats were divided into four groups of five each and a 4 cm^2 area of dorsal section of all rats was shaved. Group I (control) received no treatment. Group II (positive control) was treated with standard formulation including 1 mL of 5% minoxidil ethanolic solution applied on the shaved area once a day. Group III (blank) was treated with the prepared formulation (1 g) without eugenol applied on the shaved area once a day, and Group IV animals were treated with 1 g of prepared formulation under study once a day. All treatments lasted for 28 days. During the treatments, hair growth initiation and completion time was observed. Hair was randomly plucked from the test area of each rat on days 7, 14, and 21 of the experiment. The length of 10 hairs per animal was estimated and the average length was recorded (Table 4). At the completion of the treatments, two rats from each group were euthanized and skin biopsies were taken from shaved areas. Specimen was preserved in 10% formalin. Tissues were embedded in paraffin wax, sectioned into uniform thickness of 10 µm and stained with hematoxylin and eosin. The sections were evaluated microscopically for the number of hair follicles (24,25).

Ethical issues

All the animals were handled in accordance with the internationally accepted principles and guidelines for the care and use of laboratory animals in 2010 (26) and the Ethics Committee of Isfahan University of Medical Sciences.

Results

Authentication of eugenol

UV spectrum eugenol

As Figure 2 shows, the maximum absorption of eugenol at 5-50 µg/mL concentrations in ethanol 96% at 200-400 nm was seen at 282 nm. The UV spectrum obtained from eugenol sample in ethanol 96% solution was similar to the standard eugenol.

Self-emulsion preparation

As Table 1 shows, the best formulation was F6 containing eugenol (1%), emu oil (5%), cetrimonium chloride (0.5%),

cremophor (2%), cetyl alcohol (2%), tween 80 (1%), coconut fatty acid diethanolamin (2%) and canola oil (up to 100%). The oil phase was separately heated to 50-60°C. Then the aqueous phase (95 mL water) was added to 5 mL of oil phase with continuous stirring. When the emulsion cooled to room temperature, control tests were done.

Quality control tests of selected formulation

The F6 had light white color and a specific odor (Table 2). Microscopic examination of the prepared formulation revealed homogeneity of globule and internal phases.

Determination of particle size of the prepared emulsion

In order to reach to a particle size less than 10 µm able to penetrate into follicular structure, the average particle size of F6 in the present study was approximately 7 µm.

In-vitro release profile of eugenol from the prepared formulation

The in vitro investigation of the drug release of the formulation presented a controlled drug release for a period of 4 hours (Figure 3).

Kinetic analysis of drug release

The in vitro investigation of drug release of the formulation exhibited release for a period of 4 hours. According to the release based on the correlation coefficient, first-order kinetic is dominant.

Stability studies

According to Table 3, F6 did not show changes in color and phase separation at 8°C, 25°C and 40°C within one month. The drug content of the formulation was found to be in the range of 96.5%-98% at 8°C, 25°C and 40°C, which represents a permitted range (100 ± 5) percentage of variation (27).

Skin irritation test

Evaluation of the studied formulation for possible irritation on intact skin of rats showed no erythema or edema on outer layer of skin, indicating the safety of the prepared F6 containing 1% eugenol for topical application.

Hair growth activity evaluation

Hair growth initiation significantly increased by treatment with standard drug and the studied formulation. The hair growth was initiated in denuded area on day 15 in control rats, while it was initiated after the first week in positive control group and in the F6-treated group. However, the completion time was not affected by different treatments in this study. Table 4 shows the average hair length of each group at different time intervals during the experimental period. The average hair length significantly increased with different treatments compared to the control group.

Discussion

Self-emulsifying drug delivery systems (SEDDSs) are mixtures of oils, surfactants and co-surfactants. Sometimes co-solvents are also used to increase the solubility. Therefore, SEDDSs have also become an important tool in novel drug delivery in recent years. SEDDSs emulsify spontaneously and produce fine oil-in-water emulsions if introduced into an aqueous phase under gentle agitation (28,29).

The prepared emulsion formulation, with good characteristics based on pharmaceutical evaluation, consists of eugenol, emu oil, setrumonium chloride, cremophor, cetyl alcohol, tween 80, coconut fatty acid diethanolamine, canola oil and water.

A salient feature of this formulation is use of emu oil. Emu oil is compatible with human skin lipid and can be used as an enhancer and drug carrier to help the penetration of active ingredients through the skin.

The results showed that the effect of emulsion (F6) on hair growth began on day 6 after the treatment and commercial minoxidil 5% solution exerted its effect on

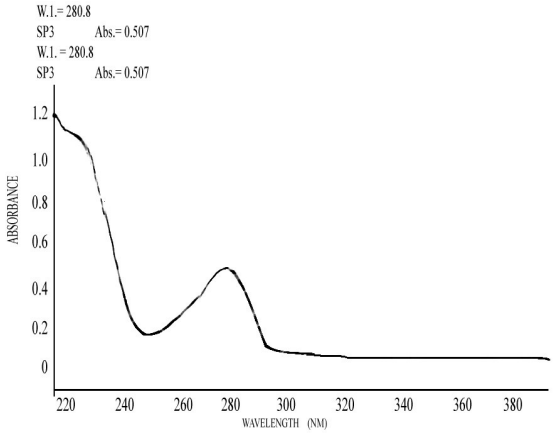

Figure 2. Eugenol standard curve in ethanol 96%.

Table 2. Physicochemical evaluation of formulation

Parameters	Results
Physical appearance	Light white color and an specific odor, complete homogeny
Centrifuge	+++
Freeze-thaw	+++
pH	5.873 ± 0.146
Drug content	98.09 ± 0.36

+: poor after test, ++: good after test, +++: excellent after test.

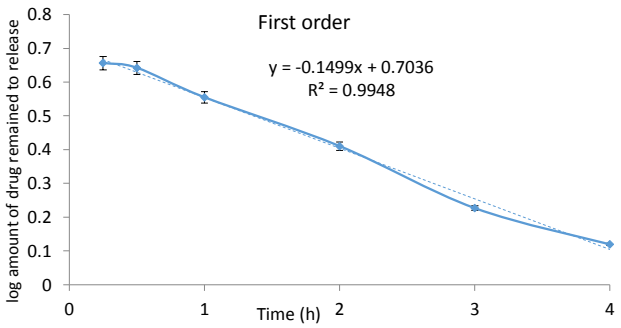

Figure 3. In vitro release of eugenol through cellulose acetate membrane.

Table 3. Stability of the studied parameters of selected formulation

Parameters	Result				
	Condition	Initial	7 days	14 days	28 days
Physical appearance					
	8 °C	√	√	√	√
	25 °C	√	√	√	√
	40 °C	√	√	√	√
Drug content (mean ±SD)					
	8 °C	98.07% ± 0.41	97.7 ± 1	97.6 ± 1.02	97 ± 1.07
	25 °C	98.07% ± 0.41	97.9 ± 1.02	97.5 ± 1.16	97.56 ± 1.16
	40 °C	98.07% ± 0.41	97 ± 1	96.88 ± 1.023	96.5 ± 1.07

√: Unchanged

Table 4. Mean (standard deviation) hair length in different groups at various intervals

Group	Day 7	Day 14	Day 21	Day 28
Control	-	-	0.445±0.089	0.57±0.115
Standard	1.08±0.113	1.58±0.122	2.16±0.183	2.5±0.163
Blank	-	0.51±0.061	0.93±0.105	1.11±0.11
Treatment	0.53±0.1	1.07±0.082	2.2±0.188	2.85±0.164

day 3 after application, but the hair growth accelerated after one week. The animals which were treated with blank formulation showed greater conversion of follicle compared with the control group on day 28 after the treatment. This may be due to the effect of emu oil on hair growth and gentle rubbing of the shaved skin area during treatment which may enhance blood flow to the hair follicles. A clinical study on the activity of emu oil on hair growth revealed that there was a 20% increase in DNA synthesis, hair and skin re-growth stimulated and hair follicles robustness increased (30). In a study on rats, when treated similarly with water, the whole denuded area was covered at the end of the treatment course because of gentle rubbing of the site (31).

Conclusion

The prepared eugenol in self-emulsion formulation was stable by different pharmaceutical evaluations. The emu oil used in this formulation could increase penetration. This formulation could be a good candidate for hair loss treatment.

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Authors' contributions

All authors contributed to the design of the study. ASH carried out the study. MAS, ASH and LS prepared and confirmed the final manuscript.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission and redundancy) were completely observed by authors.

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