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Original Article

Preparation and evaluation of minoxidil foamable emu oil emulsion

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Abstract

The aim of this work was to develop and evaluate a minoxidil foamable emu oil emulsion with the purpose of improving minoxidil permeation into the skin, increasing hair growth , reducing skin irritation, and increasing consumer compliance. Minoxidil was dissolved in a solvent system comprising ethanol: glycerin: lactic acid: water (10:20:5:65). The foamable emulsion was prepared by mixing the oil phase with minoxidil solution using different amount of various emulsifiers. Seventeen formulations were prepared and the most stable foamable emulsion was selected and evaluated for various pharmaceutical parameters such as homogeneity, pH, stability to centrifugal stress, freeze-thaw and foamability. The adopted formulation showed good pharmaceutical characteristics. In vitro release rate of the formulations were evaluated using Franz diffusion cell using phosphate buffer pH 7.4 and ethanol as the receiver medium at sink condition. The release rate of formulations was found to obey Higuchi kinetic model. Experimental animal study was performed to evaluate hair growth potential of the formulation. Different cyclic phases of hair follicles, like anagen, and telogen phases, were determined at one month period. Histological study after treatment with adopted formulation exhibited greater number of hair follicles in anagenic phase (96%) which were higher as compared to marketed 5% minoxidil solution (Pakdaru® 70%) and the control group (42%). From animal study it was concluded that the selected formulation exhibited a significant potency in promoting hair growth in comparison with marketed 5% minoxidil solution Pakdaru®.

Keywords: Hair growth; Minoxidil; Emu oil; Foamable emulsion

INTRODUCTION

Loss of hair or alopecia is one of the most common problems of many societies causing considerable economical and physiological consequences. Alopecia generally pertains to the loss of hair on the scalp, although other body sites may be affected. Androgenetic alopecia (AGA) or male pattern baldness is the most common type of hair loss in males, affecting 50% of men by the age of 50 years, and up to70% of all males in later life. AGA results from an androgenic influence on hair follicles in certain area of the scalp leading to follicular miniaturization, the process by which hair shaft become finer and shorter in an easily recognizable pattern of bitemporal and vertex thinning over several years. Terminal hairs are gradually replaced by progressively finer and less pigmented miniaturized hairs. Eventually, the follicle does not grow a new hair, however, the follicles remain alive suggesting that to the growth of new hairs is most likely (1).

There are many treatments available for regrowth of the hair of which, only two treatment shave FDA-approved indication for the treatment of AGA: minoxidil and finasteride (2). Minoxidil is the only FDA approved topical medication with proven efficacy for the treatment of AGA. Although the mechanism of action of minoxidil is unknown, it may increase the blood supply to the scalp allowing more oxygen, blood, and nutrients to the follicle which may lengthen the anagen phase by proliferative and anti apoptotic effects on dermal papilla cells of the hair follicles (3,4).

The drug is marketed as 2 and 5% topical solutions. It has been approved that 2%

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solution is less effective than 5% solution in producing the desired results. Minoxidil solution in the market contains propylene glycol and ethanol at high percentage as the main components. Most of the adverse reactions including itching, contact dermatitis and dryness are attributed to these ingredients. The tendency of minoxidil to f yield insoluble crystalline form as the ethanol evaporates through application on the skin minoxidil topical solution shows inefficient uptake by the skin. Further, minoxidil has poor skin penetration ability which limits its usefulness as a potent treatment of the hair loss (3,5).

To minimize the side effects and to improve therapeutic efficiency the new formulation of minoxidil in the form of foam is now approved for the treatment of the hair loss. Compared to other topical dosage forms, foam may provide unique properties and advantages (6). In the current study, it was attempted to develop a desirable formulation in the form of a foamable emu oil emulsion containing less amount of ethanol and devoid of propylene glycol Emu oil is one of the fastest and most penetrating oil into the skin because of its similarity to the human sebum which makes it an excellent dermal carrier getting into the scalp enhancing the potency of topical medications.

The penetrating effects of emu oil may be related to its non-phosphorus composition. Applying anything on the skin that is phosphor-lipid deficient or has no phosphorus, it penetrates right through the skin. In addition to emu oil's amazing ability to penetrate the skin, it has shown significant effect on stimulating hair growth by supplying fatty acids that are needed for hair follicle cell division. Furthermore, it naturally contains a high level of linolenic acid which can inhibit 5-alfa-reductase enzyme (7,8).

MATERIALS AND METHODS

Minoxidil (FAGRON UK Ltd), Sorbitan isostearate (Crill 6), polysorbate 80 (Croda Chemicals UK Ltd), disodium cocoamphoacetate (DSCAD) was obtained as gift sample from Afra Chemie Atis Company, Tehran Iran. Emu oil was obtained from Abyaneh cosmetic company, Isfahan Iran. All other ingredients used in this study were of analytical grade.

Authentication of minoxidil

Based on USP, BP and European pharmacopeia two methods were used to authenticate the purchased minoxidil: determination of UV and IR spectrum.

Determination of solvent system for minoxidil

The aim of this work was to decrease the amount of ethanol in the formulation and to replace propylene glycol with glycerine which is the main components of the currently marketed minoxidil solutions. Therefore, a new solvent system for minoxidil to increase minoxidil solubility was developed. (Table 1)

Preparation of foamable emu oil emulsion

In the present study, an airspray foam pump dispenser line® method was used to develop foamable oil in water emulsion, which creates foam without using propellant. This unique and patented technology allows for the precise mixing of liquid and air, resulting in a dose of high quality foam with each single stroke (6). At first, a stable emulsion was prepared and a foaming agent was then added to this emulsion which was finally transferred into the airspray pump foam dispenser. To develop a stable emulsion, several formulations were prepared using different amount of emulsifiers. The compositions of prepared formulations are shown in Table 2.

The oil and aqueous phase was separately prepared and to reach a homogeneous mixture and better emulsification both phases were separately heated to 30 - 40 °C; then the aqueous phase was added to the oily phase with continuous stirring. For further homogeneity and stability, the prepared emulsion was sonicated (Probe sonicator, Bandeline HD 3200, Germany) with power of 40 for 2 min (9). When the emulsion was cooled to room temperature, prepared DSCAD solution as the foaming agent was added with continues stirring. Based on primary evaluation of physical appearance and centrifuge test of prepared emulsions, one formulation (F17) was selected and final evaluations were performed on this formulation.

Table 1. Solvent systems designed for minoxidil.

Solvent systems	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Minoxidil (g)	5	5	5	5	5	5	5	5	5	5
Ethanol (ml)	50	50	50	50	10	10	10	10	10	10
Glycerine (ml)	-	30	30	20	20	20	20	20	20	20
Propylene glycol (ml)	30	-	-	-	-	-	-	-	-	-
Lactic acid (ml)	-	-	0.5	0.5	0.5	1	2	3	4	5
Water up to (ml)	100	100	100	100	100	100	100	100	100	100

Table 2. Minoxidil foamable emu oil emulsion formulations.

Formulations	Minoxidil solution* (6.25%) (ml)	DSCAD ** (g)	Emu oil (g)	Tween 80 (g)	Crill 6 (g)	BHT*** (g)
F_1	80	10	2	1	-	0.5-1%
F_2	80	10	2	2	-	0.5-1%
F_3	80	10	2	3	-	0.5-1%
F_4	80	10	2	4	-	0.5-1%
F_5	80	10	2	5	-	0.5-1%
F_6	80	10	2	0.3	0.7	0.5-1%
F_7	80	10	2	0.6	1.4	0.5-1%
F_8	80	10	2	0.9	2.1	0.5-1%
F_9	80	10	2	1.2	2.8	0.5-1%
F_{10}	80	10	2	1.5	3.5	0.5-1%
F_{11}	80	10	2	1.8	4.2	0.5-1%
F_{12}	80	10	2	2.1	4.9	0.5-1%
F_{13}	80	10	2	2.4	5.6	0.5-1%
F_{14}	80	10	2	2.7	6.3	0.5-1%
F_{15}	80	10	2	3.0	7	0.5-1%
F_{16}	80	10	2	4.0	6	0.5-1%
F_{17}	80	10	2	5.0	5	0.5-1%

*Table 1, solvent 80 ml system S10, **disodium cocoamphodiacetate, ***butylated hydroxyl toluene.

Evaluation of selected formulation

The following pharmaceutical parameters were used for the evaluation of formulations.

Physical appearance

The prepared foamable emulsion was inspected visually for colour and homogeneity.

Centrifuge test

The prepared formulations were centrifuged at 3000 rpm for 30 min (HETTIC D-7200, Germany) 24 h after preparation and at one week intervals for 28 days (10).

Freeze - thaw cycle

Freeze-thaw treatment of emulsion was performed 24 h after preparation. Samples (20 ml) were stored at -20 °C for 24 h. Then frozen samples were thawed at room temperature for 24 h. This test was carried out in triplicate for each sample (11).

Determination of pH

pH values of prepared formulation were measured by a digital pH meter (Metrohm-switzerland). After calibration, the determinations were carried out in triplicate and the average of three readings was recorded (10).

Determination of foamability and foam stability

Cylinder shake method was used for determining foaming ability. Fifty ml of the formulation was pured into a 250-ml graduated cylinder. The cylinder was covered and shaken for 10 minutes by wrist action shaker (Burrel BB, Canada). The total volumes of the foam immediately after shaking and at 1 min intervals for 4 min were recorded (12).

Drug content

To determine drug content, a certain amount of emulsion (10 puffs equal to one

gram of emulsion) was collected and placed 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 h 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 minoxidil was determined spectrophotometerically (Shimadzu, model UV mini - 1240CE) by measuring the absorbance of the filtrate at 289 nm. This process was performed for an emulsion system containing no drug as the blank.

In-vitro drug release study

Franz diffusion cell (25 ml volume) was used for the drug release studies. The receiver compartment was filled with phosphate buffer (pH 7.4) and ethanol at ratio of3:1which was attained after sink condition study; 10 puffs (equal to one gram emulsion) of foamable emulsion were applied on to the surface of cellulose acetate membrane. The membrane was clamped between the donor and the receiver compartment. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37 °C. 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 immediately replaced with fresh 0.5 ml of receiver medium. After suitable dilution the drug concentration on the receiver medium was determined spectrophotometerically at 289 nm. The experiment was carried out in triplicate (13). To study drug release kinetics, data obtained from in vitro release study were fitted to the zero, first and Higuchi kinetic models. In order to evaluate mechanism of drug release, data of drug release were fitted to the Korsmeyer-Peppas equation (14).

Stability tests

The stability studies were carried out at 8 °C (at refrigerator), 25 °C (at room temperature), 40 °C (in an oven) and 4 0°C with 75% relative humidity (again in an oven). At one week intervals for one month, drug content and physical appearance (organoleptic characteristic) were evaluated (9).

Animal study

Female Wistar albino rats, 200 ± 25 g, and age 8 ± 1 months were used for hair growth studies. Based on experimental design the animals were divided into 5 groups of 6 rats each. Each group was placed in individual cages and kept in standard environmental conditions, the rats were fed with rat pellets and water ad libitum. All animal experiments were carried out in accordance with guidelines of Institutional Animal Ethical Committee of Isfahan University of Medical Sciences.

Skin irritation test

The rats were divided into 5 groups of six rats each. A 4 cm^2 area of dorsal portion of all rats were shaved and wiped with surgical spirit. About 10 puffs of the formulation were applied over the site. The test sites were observed for erythema and oedema for 48 h after application (15).

Application of test formulations for hair growth evaluation

The rats were divided into 5 groups of 6 rats each. A 4 cm^2 area of dorsal portion of all the rats was shaved off to remove all hair. Group I was kept as control which received no drug treatment. Group II was treated with marketed formulation, where 1 ml of (5% minoxidil ethanolic solution) was applied over the shaved area, once a day. Group III was treated with prepared formulation without minoxidil as the blank, where 10 spray of formulation was applied over the shaved area, once a day.

The animals of remaining groups were given application of 10 sprays of prepared formulation once a day. This treatment was continued for 30 days. Skin biopsies were taken from shaved areas and specimen was preserved in 10% formalin. Tissues were embedded in paraffin wax and sectioned into uniform thickness of 10 μm and stained with hematoxylin and eosin. Sections from all the groups were evaluated for the number of hair follicles per mm area of the skin and percentage ratio of hair follicles in different cyclic phases, like anagen and telogen was determined microscopically (16).

RESULTS

Authentication of minoxidil

FTIR spectrum of minoxidil

The principle peaks of IR spectrum of minoxidil presented in British pharmacopeia are at wave numbers 1640 cm^{-1}, 1610 cm^{-1}, 1550 cm^{-1}, 1231cm^{-1}, 1210 cm^{-1} and 758 cm^{-1}. All the above characteristic peaks of standard minoxidil appeared in obtained spectra of used minoxidil and were in agreement to those reported in the British pharmacopeia (Fig. 1).

UV spectrum of minoxidil

The maximum absorbance of minoxidil in aqueous alkali solution was seen at 262, 288 nm and in aqueous acidic solution at 230, 281 nm.

The UV spectrum obtained from purchased minoxidil in both aqueous acid and alkali solutions was similar to that reported in the British pharmacopeia. (Fig. 2).

Development of solvent system

According to Table 1, S1 is the solvent system of minoxidil topical solution in the market. When propylene glycol replaced with glycerine and the amount of ethanol decreased to 10%, solubility of minoxidil intensively decreased. To overcome this problem, lactic acid as the co-solvent was added to subsequent formulations (S3 - S10). Among these working solvent systems, in S2 and S5 the desired amount of minoxidil was not dissolved. Minoxidil was initially dissolved in S4, S6, S7

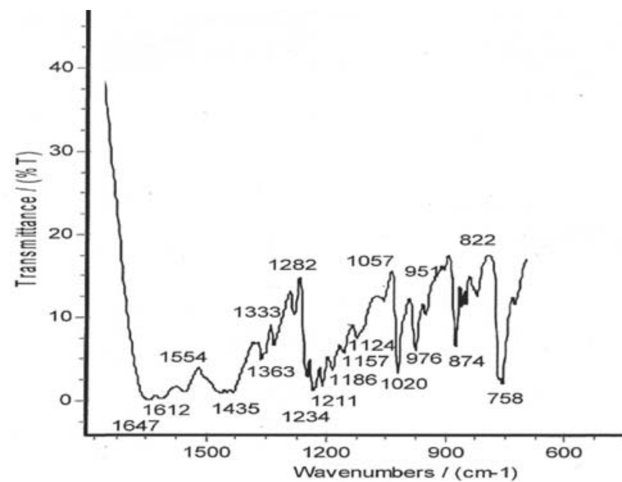

Fig. 1. FTIR spectrum of minoxidil.

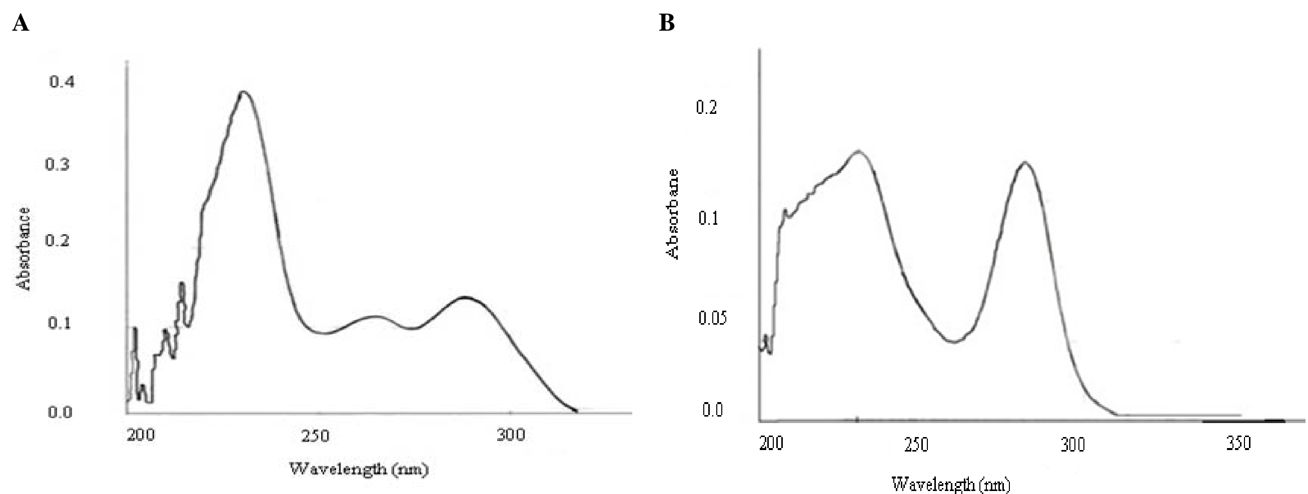

Fig. 2. UV spectrum of minoxidil (A) in alkali solution (λ_{max}= 262.2, 288.3), (B) in acidic solution (λ_{max}= 231.2, 281).

and S8 solvent systems, but after 0.5-1 h its solubility was decreased and crystal formation and instability was observed. S1, S3, S9 and S10 completely dissolved minoxidil and solutions were clear with no crystal based on centrifugal and visual analysis. Amongst these solvent systems, S10 was considered more appropriate as minoxidil even above 5% could be dissolved.

Foamable emulsion preparation

According to Table 2, formulations containing only 1-5% tween 80, (F1-F5) were not stable and the phases were separated immediately after preparation. Therefore, to obtain stable emulsion various amount of Crill 6 was added as seen in the formulations F6-F17. The appropriate RHLB of the oil phase-containing emu oil is reported to be around 8, so RHLB needed for a stable emulsion was considered to be around this value. The ratio of tween 80 to crill 6 at 0.3:0.7 in this system provides the required HLB of about 8(17). In formulation F6, overall one percent of surfactant was used but did not result in a stable system. In subsequent formulations, therefore, amount of surfactant was increased gradually from 1 % to 10%. (F7-F17). Amongst F7–F15 formulations, F15 was considered stable, in which the required amount of emulsifiers need to obtain a stable emulsion was 10% (F15-F17). Since Crill 6 is a viscose liquid, its higher amount in the formulations will result in a viscous emulsion. Thus in formulations F16 and F17 it was attempted to reduce the amount of Crill 6. Collectively, formulation F17 was found to be most appropriate one based on physical assessments.

Quality control examinations of adopted formulation

The selected formulation (F17) was completely homogeneous in appearance, having white to cream color and specific odor. Observation of prepared formulation under the microscope revealed homogeneity of globules of the internal phase. pH values of 1% (w/w) solution of selected formulation was in the range of skin' pH. Thus, this formulation is appropriate for application on the skin surface, as the pH of subcutaneous and upper viable epidermis is evaluated to be about 4 (Table 3).

Evaluation of foamability and foam stability

Foam generation and stability could have more consumer acceptability and to achieve these criteria we designed a formulation to be stable for at least 5 min after application on the scalp and before applying shear force (Fig. 3).

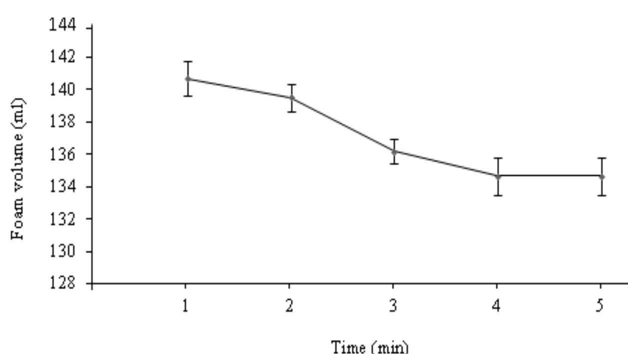

Fig. 3. Foam retention profiles of selected formulation (F17).

Table 3. Physico-chemical evaluation of selected formulation (F17).

Parameters	Results
Physical appearance	White to cream in colour , completely homogeneous
Centrifuge	+++
Freeze-thaw	+++
pH	4.15 ± 0.136
Drug content	98% ± 0.120

+: poor, ++: good, +++: excellent.

Drug release kinetic analysis

The in vitro drug release study of the formulation exhibited a controlled drug release for a period of 6 h (Fig. 4). According to the release exponent (0.45<n<0.89) diffusion mechanism is non- Fickian, and based on the correlation coefficient Higuchi kinetic is dominant (Table 4).

Stability studies

According to Table 5, formulation F17 did not show changes in colour and phase separation at 8 °C and 25 °C during one month, but there was slight phase separation at 40 °C after 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 is in the normal (100 ± 5) permitted range of variation (18).

Skin irritation test

Skin irritation test was conducted to evaluate the irritation by the prepared formulation on intact skin of rats. The prepared formulation did not show any erythema or oedema; this indicates that the prepared formulation (F17) containing 5% lactic acid was non-irritant on skin of rats.

Hair growth activity evaluation

The histological appearance of the skin in treated groups was similar with that of the control group. The number of hair follicles per mm of the skin was found to be 10 ± 2 in all groups. However, there was a marked difference in the cyclic phases of the hair follicle (anagen and telogen) between studied groups (Fig. 5). According to Table 6 and also Fig. 6, on day 10 after the treatment, percent of follicles in anagen phase was increased significantly in the group receiving adopted formulation (63%, P<0.05) as compared to the control, but in the other groups there was no significant increase in anagen follicles (P>0.05). On day 20 after the treatment, adopted formulation as well as the market formulation showed considerable increase in anagen follicles. On 30th day adopted formulation showed maximum number of anagen hair follicles (96%) in comparison with the control group, and rats receiving blank and market formulations; the market formulation group also produced higher value (70%) than blank (48%) and control (42%), but to a lesser extent than adopted formulation.

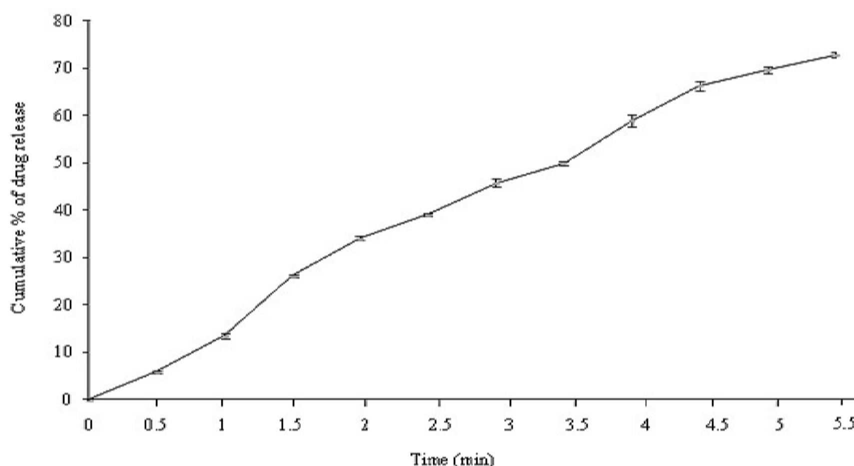

Fig. 4. In vitro release of minoxidil through cellulose acetate membrane from selected formulation (F17).

Table 4. Kinetic parameters of selected formulation release.

Zero-order		**First-order**		**Higuchi**		**Korsmeyer-Peppas**		
R^2	**k (h^{-1})**	**R^2**	**k (h^{-1})**	**R^2**	**k ($h^{-1/2}$)**	**R^2**	**k (h^{-n})**	**n**
0.982	0.0724	0.986	0.2572	0.992	42.9300	0.981	0.0981	0.886

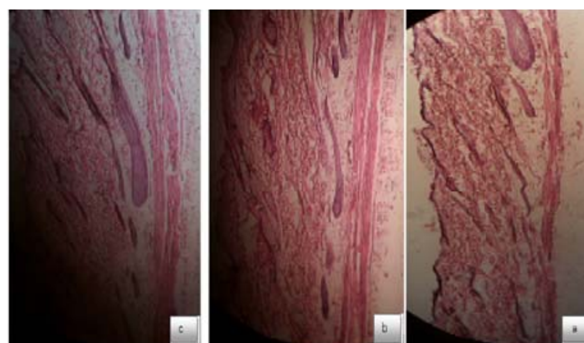

Fig. 5. Skin Histology section of Albino rats (a) control, (b) market formulation, (c) main formulation (10x).

Fig. 6. Albino rat skin treated with adopted formulation at day 20 (A) and at day 30 after the treatments (B). Marketed 5% minoxidil solution at day 20 (C) and at day 30 after the treatments (D). Blank formulation at day 20 (E) and at day 30 after the application (F). No drug as control group at day 20 (G) and at day 30 after application to the skin (H).

Table 5. Stability study parameters of selected formulation.

Parameters	Condition	Initial	7 days	14 days	28 days
Physical appearance	8 °C	+++	+++	+++	+++
	25 °C	+++	+++	+++	+++
	40 °C	+++	+++	+++	+++
	40 °C/75% RH	+++	+++	+++	+++
Drug content (mean ± SD)	8 °c	98% ± 0.12	97.7 ± 1	97.6 ± 1.02	97 ± 1.07
	25 °c	98% ± 0.12	97.9 ± 1.02	97.5 ± 1.16	97.56 ± 1.16
	40 °C	98% ± 0.12	97 ± 1	96.88 ± 1.023	96.5 ± 1.07
	40 °C/75% RH	98% ± 0.12	97 ±1.001	97 ± 1.1	96.74 ± 1.08

+: poor, ++: good, +++: excellent

Table 6. Hair growth activity evaluation of minoxidil foamable emu oil emulsion.

Applied formulation	Percentages of hair follicles (mean ± SEM)					
	Day 10		Day 20		Day 30	
	Anagen	Telogen	Anagen	Telogen	Anagen	Telogen
Control	34 ± 0.3	66	37 ± 0.7	63	42 ± 0.9	58
Market formulation (minoxidil solution 5%)	36 ± 0.9	64	56 ± 0.3***	44	70 ± 0.9***	39
Blank formulation	34 ± 0.3	66	39 ± 0.3	61	48 ± 0.3****	52
Main formulation	63 ± 1.1*	47	88 ± 0.9**	12	96 ± 0.7**	6

Values are expressed as mean ± SEM. Statistical significance was determined using paired-sample t-test. Values with (P<0.05) were considered significant. *P=0.002, **P=0.001, ***P=0.003, **** P=0.035 (P<0.05) compared to control group by paired-sample t-test.

DISCUSSION

Pharmaceutical foams are not new inventions and their application in topical therapy can be traced back three decades. The use of foam in dermatology was first reported in 1977 by Woodward and Berry, who studied the therapeutic benefits of betamethasone benzoate hydroalcoholic quick-break foam in comparison with its semi-solid dosage form. Their study showed high clinical efficacy and excellent patient acceptability in the treatment of psoriasis (19).

Purdon and co-workers in their study revealed that in comparison with other topical vehicle such as creams, lotions, gels and ointments, foam vehicle improved patient compliance. The real reason for the rapid growth of foam technology in dermatology is that foams are elegant, aesthetic and cosmetically appealing vehicles, which are generally easier to apply and spread more easily than other topical vehicles (20). For example, the work of Kahanek and co-workers also revealed that desonide foams increased patient compliance in the treatment of steroid-responsive dermatoses (21). In another study, Tamarkin and co-workers evaluated the usability profile of foam against a cream control and concluded that foam was significantly better than cream control with regard to the ease of application and uniform spreading (22).

There are two main forms of topical minoxidil preparations for the treatment of AGA, liquid formulation and foam formulation; in "consumer use studies", minoxidil foam vehicle was rated significantly higher on several aesthetic attributes compared with minoxidil solution, including; ease of application, lack of dripping, quick absorption and drying, and ability to fit easily into a daily routine. Many patients prefer foam versus a solution that may drip on application to the scalp (23). To best of our Knowledge, a foamable emulsion formulation containing emu oil and minoxidil to treat AGA has not so far been studied or reported. One of the novel features of this formulation is using emu oil. Emu oil is compatible with human skin lipid and can be used as an enhancer and drug carrier to help the permeation of active ingredient through the skin. Also in this study airspray foam pump dispenser line® method was used which creates foam without using propellant and this is another novel feature of this formulation in comparison with other topical foam dosage forms. This is because propellant technology causes increasing overall cost of the product; this is the major disadvantage of topical foam formulations (6).

To minimize the side effect and to improve therapeutic efficiency the new formulation of minoxidil in the form of foamable emulsion which is propylene glycol-free and has emu oil and less amount of ethanol was prepared. When the amount of ethanol decreased and propylene glycol was replaced with glycerin, solubility of minoxidil decreased intensively, so lactic acid was added to the solvent system to increase minoxidil solubility most likely through the formation of the minoxidil lactate salt which may exhibit enhanced solubility and improve the ability to incorporate increased amounts of the active component in the composition (24).

The prepared foamable emulsion formulation consist of minoxidil, water, ethanol, glycerine, tween-80, lactic acid, crill-6 and emu oil showed good characteristics based on pharmaceutical evaluation. There was no change in colour and no phase separation observed at 8 °C and 25 °C during one month storage, however, there was a slight phase separation at 40 °C after 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 is in the acceptance range of variation. The formed foam was stable for about 5 min before application of mild shear forces. On this basis, it is categorized as “breakable” foam which is ideal for the use in dermatological and mucosal tissue applications (6). According to the release exponent ($0.45<n<0.89$), diffusion mechanism was considered non fickian which refers to a combination of both diffusion and erosion controlled release rates. Based on the correlation coefficient of release kinetics, Higuchi model is dominant which is applicable to drug release by diffusion model, where the rate controlling step is the process of diffusion

from the matrix (14). It is assumed that applying shear force after application of topical and dermal dosage forms may change the release mechanism of active ingredient because this force may cause breaking the structure of emulsion resulted in facilitation of drug release; so indeed the drug release mechanism of topical dosage form may differ from the mechanism determined by in vitro release where no shear force is applied. The histological study indicated that the conversion of telogen to anagen follicles increased in the groups treated with adopted formulation as well as market formulation when compared to the control.

The results showed that the effect of foamable emu oil emulsion on hair growth began at the day 10 after the treatment as compared with the control ($p<0.05$), but marketed 5% minoxidil solution showed its effect at 20th day after application, which could be due to emu oil's penetration enhancing effect which increases the follicular penetration of minoxidil, and its effect on hair growth which may cause synergic effect. A clinical study by Holick on the hair growth activity of emu oil revealed that there was a 20% increase in DNA synthesis, stimulated hair and skin re-growth and also hair follicles were more robust (25).

The animals which was treated with blank formulation was also showed greater conversion of follicle compared with the control group on 30th day after the treatment. This may be due to the hair growth effect of emu oil and gentle rubbing of the shaved skin area during treatment which may enhance blood flow to the hair follicles. In one study by Adhirajan and co-workers it was claimed that the rats when treated similarly with water, the whole denuded area had been covered at the end of the course because of gentle rubbing of the site (16).

CONCLUSION

The pharmaceutical study of foam dispenser emulsion containing emu oil compatible with sebum without any harsh ingredient was developed. The selected formulation was pharmaceutically stable with excellent spreading ability over the site of action leading to the promotion of patient compliance. On animal study, minoxidil formulated in emu oil significantly improved hair growth, as compared to Pak-Daru® 5% minoxidil solution, a product widely marketed in Iran. Our formulation was well compatible with skin and caused no sensitivity reaction in the animal model used. Further studies in human are, however, needed to confirm its suitability and safety profile in human.

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