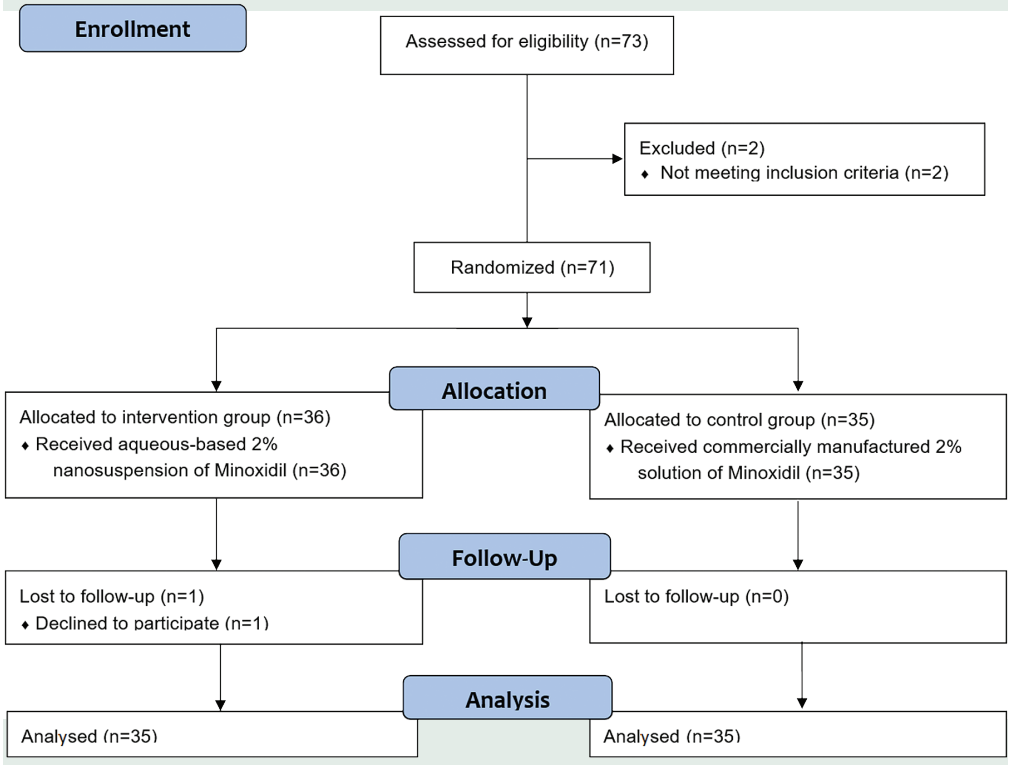
Original Article

A Randomized, Double-blind Controlled Clinical Study to Evaluate the Efficacy and Safety of Minoxidil Topical 2% Nanosuspension with Aqueous Base in the Treatment of Androgenetic Alopecia Areata

Abstract

Background and Purpose: Using the commercially manufactured forms of minoxidil, the only approved topical drug preparation for hair regrowth in patients with androgenetic alopecia (AGA) comes across with challenges such as limited permeation through the superficial layers of the skin to reach the site of action and topical adverse reactions like itching and inflammation occur because of the ethanol in the formulations. In this study, a novel nanosuspension formulation with an aqueous base was prepared and evaluated to overcome the discussed challenges. **Materials and Methods:** The nanosuspension formulation was characterized by size, zeta potential, morphology, and *in vitro* release. Seventy patients were subjected to use either 1 mL of nanosuspension or the commercial product twice daily for six months and were then examined for changes in hair follicle diameter and hair density within a 1 × 1-cm^2 area of the scalp as the primary endpoints besides any adverse reaction manifestation as the secondary endpoint. **Results:** The nanosuspension formulation showed uniform morphology, 200-nm particle size, and suitable zeta potential that ensures the stability. The *in vitro* release study exhibited almost 90% release in the first 6 h. It was observed that there were no significant differences between the efficacy of aqueous-based topical 2% nanosuspension of minoxidil and the commercial product in the treatment of AGA ($P > 0.05$). However, the aqueous-based topical 2% nanosuspension formulation showed better safety and tolerability compared to the marketed profile. **Conclusions:** It could be concluded that aqueous-based topical 2% nanosuspension is a suitable form with enhanced patient compliance compared to commercially manufactured products.



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Keywords: *Androgenetic alopecia areata, clinical study, minoxidil, nanosuspension*

Introduction

Hereditary hair loss is a condition in which the overall amount of scalp hair coverage in vertex and frontotemporal areas reduces by aging, presumably due to the shortening of hair growth cycle length. It is also called androgenetic alopecia (AGA), which is a common reason for hair loss in adults, especially in males. Based on the previous reports, it affects almost 50% of the population with the age over 50 years.[1,2] It was reported that it also affects 6%–38% of healthy females.[3] The reason behind the importance of treatment in these patients is the great discomfort, isolation, and reduction in quality of life this disease could cause.[4] There is only one Food and Drug Administration (FDA)-approved topical agent for the treatment of AGA, which is the topical preparation of minoxidil.[5,6]

Minoxidil is a vasodilator and is orally administered in hypertensive patients, which is also categorized as a topical skin product. Following the FDA approval of oral minoxidil in 1988, it was observed that minoxidil could cause hypertrichosis. Nowadays, topical minoxidil solution, which is formulated using purified water, ethanol, and propylene glycol, is approved by the FDA for the stimulation of hair regrowth in patients over 18 years of age.[7] The mechanism of minoxidil in stimulating hair growth is still uncertain, but it seems that minoxidil acts by affecting different phases of hair growth cycles. It is reported that minoxidil also causes hair regrowth by the opening of the potassium channels and increasing vascular endothelial growth factor synthesis in dermal papillary cells.[3]

Because of the barrier function of the skin and stratum corneum, most of the topical skin products have limited absorption and permeation through the superficial layers of the skin toward their site of action.[8] Using nanosuspension formulation can enhance the transdermal delivery of drugs because of the smaller size of particles and also the increased surface-to-volume ratio.[9-11] Most of the commercially manufactured minoxidil preparations face complications like topical skin reactions including erythema, itching, and inflammation, which occur due to the existence of ethanol in their formulation.[12] This product has the advantage of being aqueous-based, which could extremely reduce the topical reactions of the product compared to the other formulations.

Previous studies showed an improved topical delivery resulted from the formulation of minoxidil into a nano-based delivery system. Matos *et al.*[13] designed and developed chitosan-based nanoparticles for targeting minoxidil to hair follicles, which reported a prolonged release and enhanced permeation through the skin. Minoxidil-loaded methylcellulose nanoparticles were designed and evaluated in a study. The results confirmed the improved efficacy of nanoparticles compared to the commercial solution due to the higher drug contents in the hair bulbs of rats.[14] In a previous study, minoxidil-loaded nanoemulsions prepared using oleic acid or eucalyptol showed an enhanced permeation and retention through the skin.[15]

In this study, the efficacy and safety of aqueous-based topical 2% nanosuspension of minoxidil were evaluated in patients by observing the changes in hair follicle diameter, hair density, and the overall coverage of scalp along with the occurrence of adverse reactions during six months. The obtained data were finally analyzed using SPSS 25.0 software.

Participants and Methods

Characterization of nanosuspension

Size and zeta potential analysis

Nanosuspension formulation was a self-designed formulation under patent application, which was provided by Rahesh Daru Novin Co. (Kermanshah, Iran). To evaluate the mean particle size, zeta potential, and polydispersity index of the formulation, the formulation was analyzed using a zeta-sizer (Malvern Instruments, Malvern, UK). The investigation was performed in triplicate, and an average was taken.

Morphology analysis

The nanoparticles were extracted by optima-L-90K (Beckmann-Coulter®, USA) ultracentrifuge device at 40,000 rpm for an hour and then frozen and lyophilized using alpha 2-4-LSC freeze dryer (Martin Christ, Germany). Scanning electron microscopy (SEM) was utilized after gold coating of formulation under vacuum condition and 5 kV accelerating voltage using KYKY-EM 3200 (KYKY, China) microscope.

In vitro release study

A certain amount of formulation was placed inside a cellulose dialysis bag with 12,000 cutoffs, as the donor compartment, and was then placed in 50 mL of phosphate buffer as the receptor compartment (pH = 7.4) under 50 rpm stirring at 37.0 ± 0.5°C temperature. The samples were withdrawn at certain time intervals with the replacement of an equal volume of receptor medium. The amount of released drug was estimated using ultraviolet–visible (UV–Vis) spectroscopy at the maximum absorption wavelength of 203 nm (n = 3).

Clinical study design

This study was carried out as a randomized, double-blind controlled trial to investigate the safety and efficacy of novel topical aqueous-based nanosuspension of minoxidil compared to commercial 2% minoxidil topical solution (Pak Darou® Pharmaceutical Co, Iran) for six months. The clinical procedure was approved by the Local Animal Ethical Committee of Kermanshah University of Medical Sciences with an approval number of IR.KUMS.REC.1397.138.

Participants

Males and females aged between 19 and 55 years in a good health condition diagnosed for AGA classified as class II to VI of the Norwood-Hamilton classification (males) and type I to III

of the Ludwig scale (females) were subjected to this study. The study was approved by the Iranian Registry of Clinical Trials on July 10, 2018 (registration number: IRCT20180120038450N1).

Inclusion and exclusion criteria

Following items were considered as inclusion criteria: age between 18 and 55 years, diagnosis of AGA, class 1–4 of the Fitzpatrick Classification for Skin Type, class II to VI of the Norwood-Hamilton classification hair loss for males, and type I to III of the Ludwig scale hair loss for females. The exclusion criteria include pregnancy and lactation, use of topical minoxidil in the last six months, use of finasteride, antiandrogenetic, estrogen, progesterone, tamoxifen, anabolic steroids, any potent medications for hyperkeratosis, oral glucocorticoid, lithium, and phenothiazines within three months before the study, hair transplantation, and diagnosis of diseases that cause hair loss like vascular disease, acquired immunodeficiency syndrome, polycystic ovary syndrome, hypothyroid, and lichen planus.

Intervention and outcome

Participants were randomized to control and intervention groups (each contain 36 patients). Participants in control and intervention groups used and massaged either 1 mL of Pak Darou® solution or minoxidil nanosuspension to the affected area of the dry scalp, twice daily. The participants were advised not to rinse their hair in the first 4 h of administration. The changes at baseline in hair diameter and density within a 1-cm^2 area of the scalp and visual scoring of total changes in scalp coverage during six months were considered as primary endpoints. The visual observations of changes in scalp coverage were evaluated by three blinded investigators, and the hair changes at baseline in participants were rated by a five-point scale, which is classified as −2 = moderate deterioration, −1 = mild deterioration, 0 = no changes, 1 = mild improvement, and 2 = moderate improvement.[6] The exact location at scalp for investigation of the hair density, diameter, and coverage changes was determined using the intersection of three anatomical points including nose tip, left tragus, and right tragus besides a self-designed 3 × 3 cm^2 transparent plastic scale. The secondary endpoints (adverse effects) including erythema, burning, itching, papule, urticaria, and folliculitis were assessed using both investigator and patient reports, compared to the baseline.

Sample size

The sample size was calculated based on the previous data, which is available based on the efficacy of the minoxidil solution. The placebo effect was considered to be 10 hairs/cm^2, with a 0.05 level of significance and 80% power; the sample size for this two-sided study was estimated to be 31 for each group.[16,17] Based on the estimated dropout rate, 36 patients were subjected to each group.

Randomization and blinding

Each patient received a random number generated by Excel (2019 version). The numbers were allocated equally to the control and treatment groups. The information that represented the study group for each patient was put in sealed, subsequently numbered envelopes. All investigators were blinded to the treatment except a statistician who was not involved in the data evaluation. The minoxidil solution and nanosuspension were filled in the same containers.

Data analysis

All data obtained for primary and secondary endpoints were analyzed using a *T*-test against the test value (placebo effect = 10 hair/cm^2) (significance level = 0.05). The SPSS 25.0 software was used for data analysis.

Results

Characterization of nanosuspension

The mean particle size and PDI of the formulation were measured to be 203 ± 39 nm and 0.230 ± 0.066, respectively. The mean zeta potential of −16.3 ± 0.5 mV was observed for the nanosuspension. The lyophilized formulation showed a mean particle size of almost 200 nm and a uniform structure after SEM analysis. The results of *in vitro* release study indicated a sustained-release profile during 24 h with a burst release in the first 6 h of releasing 87.91 ± 1.92% of the drug [Figure 1].

Baseline characteristics

Initially, 73 patients were enrolled but three patients dropped out through the study [Figure 2]. Seventy adults with AGA have completed the study including 15 females and 55 males. Age ranges were between 19 and 55 years, and the average was calculated to be 26.02 ± 7.76 years. The intervention group and the control group had an average age of 25.60 ± 6.77 and 26.45 ± 8.72, respectively. The distribution of different classes of AGA in the Hamilton-Norwood and Ludwig scales along with data related to family history and hair loss duration is represented in Table 1. The distribution of all the baseline characteristics including age, family history, hair loss scale, and duration was similar in the intervention and control groups based on the results obtained from the Chi-square test ($P > 0.05$).

Total hair diameter changes at baseline

Both formulations could increase the average hair diameter after 24 weeks of treatment. The mean change in hair diameter at baseline and after 24 weeks in the intervention group was 2.22 ± 3.00 µm, while it was 1.31 ± 2.66 µm in the control group. Although this parameter was slightly higher in the case of nanosuspension formulation than in the marketed formulation, there was no statistically significant difference between the control and intervention groups ($P > 0.05$). The data corresponding to the changes in hair diameter at baseline and after 24 weeks are presented in Table 2.

Total hair count changes at baseline

The data obtained on the total hair count changes at baseline and after 24 weeks in control and intervention groups are presented

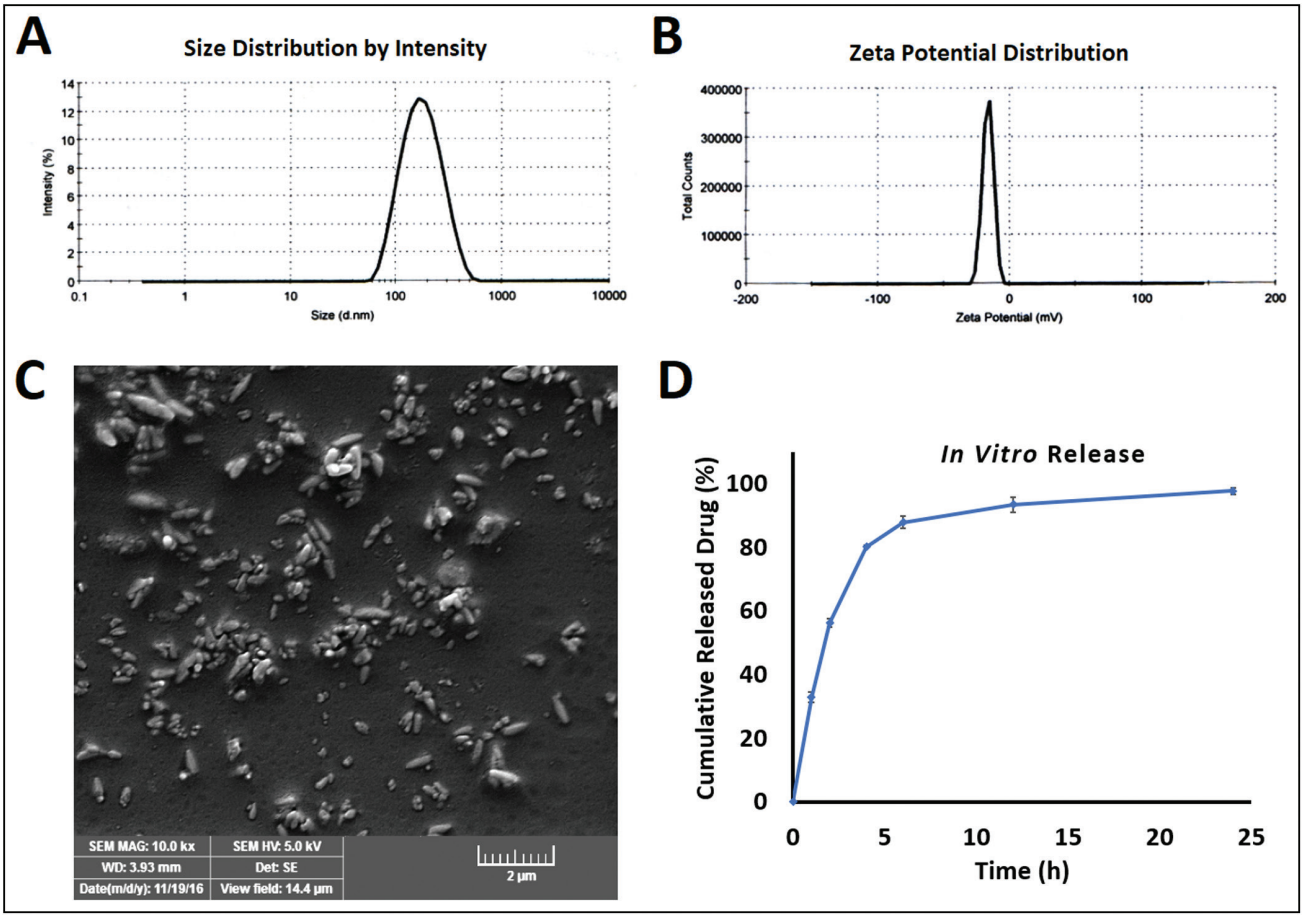

Figure 1: The physicochemical characteristics of nanosuspension formulation. (a) Size distribution; (b) zeta potential distribution; (c) scanning electron microscopy; (d) *in vitro* drug release

in Table 3. As it is obvious, despite the increased total hair count at the targeted area at baseline in both groups, no statistically significant difference was observed between the intervention and control groups ($P > 0.05$). The intervention group possessed 11.02 ± 7.76 hairs/cm^2 mean changes at baseline in the total hair count, while this parameter was investigated to be 9.82 ± 6.81 hairs/cm^2 for the control group.

Visual scoring of changes in scalp coverage

Based on the visual observations of blinded investigators, 17 (48.57%) subjects from the intervention group and 18 (51.42%) subjects from the control group did not show any significant change in the total coverage of the scalp. Fifteen (42.85%) subjects in the study group and 12 (34.28%) subjects in the control group were reported to show an improvement in the total coverage of the scalp, while three (8.57%) subjects in the intervention group and four (11.42%) subjects in the control group showed deterioration [Figure 3]. There was not any significant difference between intervention and control groups in the visual score corresponding to changes in the scalp coverage.

Safety and tolerability

Generally, fewer patients complain of topical adverse reactions in the group treated with the aqueous-based nanosuspension of minoxidil compared to the group treated with the marketed product. A total of 31 complaints of topical adverse reactions were reported during the study. Among these reported complaints of adverse reactions, 25 cases were found to be related to the use of both minoxidil commercial solution and nanosuspension. Nineteen cases of these 25 recorded complaints were detected in the patients who belonged to the control group, while six cases were detected in the patients who belonged to the intervention group. Table 4 represents the details. Figure 4 compares the prevalence of topical adverse reactions in the intervention and control groups.

Discussion

AGA is a common reason for the overall reduction in the amount of vertex and frontotemporal hair coverage. The most common treatment of this condition is the topical administration of minoxidil solution twice daily for six months.[5,6] Unfortunately, because of the existence of ethanol in the formulation, many commercially manufactured products can cause a wide variety of topical skin reactions.[12] To our knowledge, for the first time in this article, the therapeutic effects of an aqueous-based nanosuspension formulation of minoxidil on the total coverage and hair count in the AGA patients were evaluated. Also, the

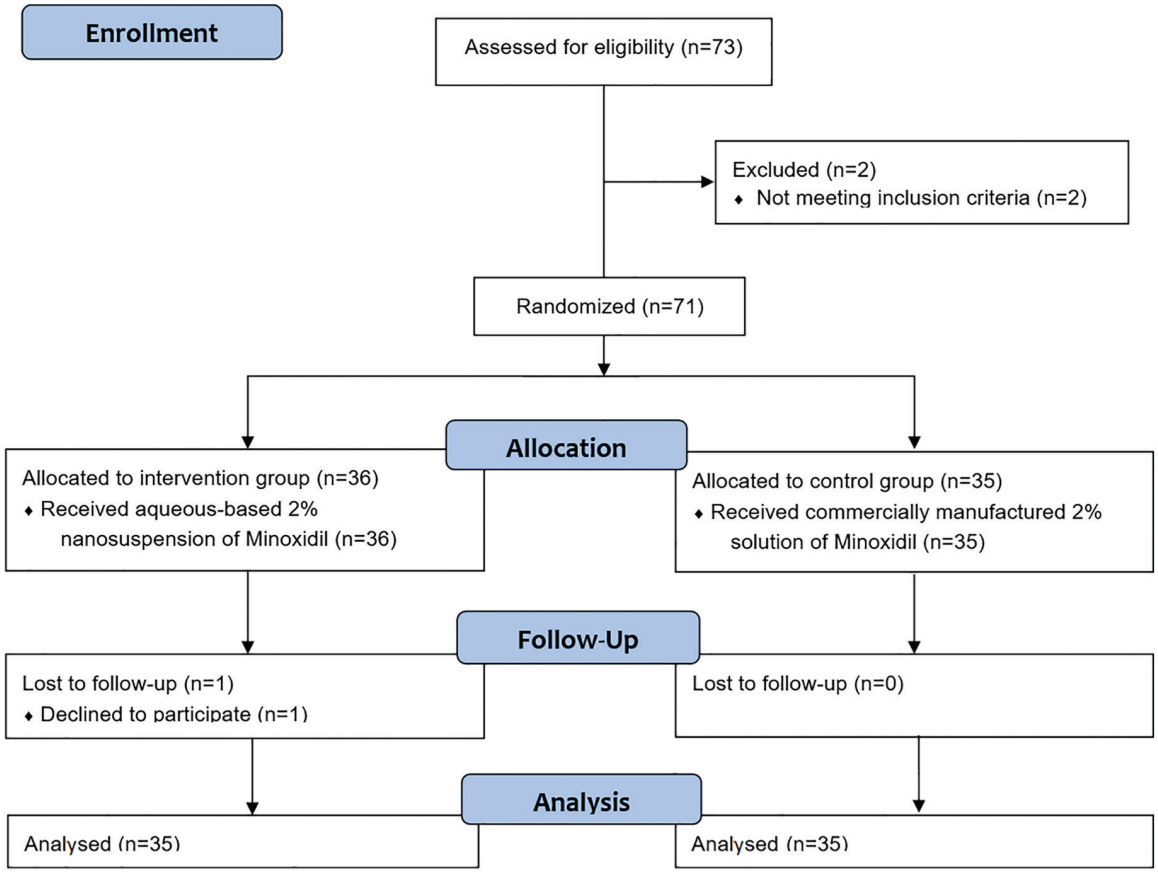

Figure 2: CONSORT 2010 flow diagram of the study

Table 1: Baseline characteristics of subjects

Parameter	All randomized subjects (n = 70)	Intervention group (n = 35)	Control group (n = 35)
Age			
Mean ± SD	26.02 ± 7.76	25.60 ± 6.77	26.45 ± 8.72
Median	23	23	23
Min–max	19–55	19–51	19–55
Hamilton-Norwood scale	**Males (n = 55)**	**Males (n = 28)**	**Males (n = 27)**
II	35 (63.6%)	17 (60.71%)	18 (66.6%)
III	20 (36.3%)	11 (39.28%)	9 (33.3%)
Ludwig scale	**Females (n = 15)**	**Females (n = 7)**	**Females (n = 8)**
I	13 (86.6%)	6 (85.71%)	7 (87.5%)
II	2 (13.3%)	1 (14.28%)	1 (13.5%)
Family history			
Mother–father	44 (62.8%)	23 (65.7%)	21 (60%)
Brother–sister	17 (24.2%)	8 (22.8%)	9 (25.7%)
Uncle–aunt	15 (21.4%)	8 (22.8%)	7 (20%)
Hair loss (years)			
Mean ± SD	1.8 ± 1.46	1.87 ± 1.74	1.74 ± 1.13
Median	1	1	1
Min–max	0.5–8	0.5–8	0.5–5

product was compared to the commercially manufactured product for topical adverse reactions.

Nanosuspension formulation was a self-designed formulation under patent application, which was provided by Rahesh Daru Novin Co. (Kermanshah, Iran). The mean particle size and PDI of the formulation were in the suitable range defined for nanosuspensions. The high negative zeta potential stabilized the formulation due to electrostatic repulsion. The SEM image demonstrated a uniform structure, and the *in vitro* release study indicated a suitable sustained-release profile with a burst release in the first 6 h. The burst release occurred due to the

Table 2: The statistical analysis of measured hair width at a 1 × 1 cm² area on the scalp at the baseline and after 24 weeks

Parameter	Intervention group (n = 35)		Control group (n = 35)		P value of intervention group versus control group
	Width (μm)	Changes from BL	Width (μm)	Changes from BL	
BL		-		-	-
Mean ± SD	53.94 ± 6.51		52.71 ± 7.45		
Median	55		53		
Min, max	42, 69		38, 74		
24 weeks					0.164
Mean ± SD	56.17 ± 6.59	2.22 ± 3.00	54.02 ± 6.73	1.31 ± 2.66	
Median	57	2	55	2	
Min, max	45, 71	−3, 8	40, 72	−3, 9	

BL = baseline

Table 3: The statistical analysis of hair count at a 1 × 1 cm² area on the scalp at the baseline and after 24 weeks

Parameter	Intervention group (n = 35)		Control group (n = 35)		P value of intervention group versus control group
	Count (number)	Changes from BL	Count (number)	Changes from BL	
BL		-		-	-
Mean ± SD	163.60 ± 19.24		163.17 ± 19.60		
Median	165		165		
Min, max	128, 202		132, 203		
24 weeks					0.523
Mean ± SD	174.62 ± 17.56	11.02 ± 7.76	173.00 ± 17.98	9.82 ± 6.81	
Median	178	12	171	11	
Min, max	132, 202	−4, 26	132, 214	−4, 23	

BL = baseline

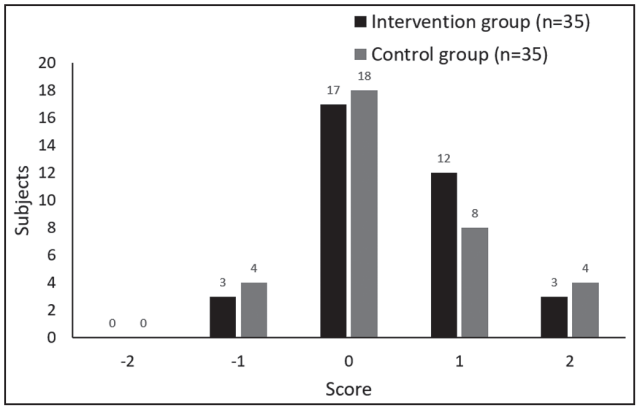

Figure 3: Comparison of intervention and control groups' scores of changes in scalp coverage obtained by the visual scoring system (−2 = moderate deterioration, −1 = mild deterioration, 0 = no changes, 1 = mild improvement, 2 = moderate improvement)

release of surface-loaded drug in the nanoparticles, and the sustained phase arises from the diffusion of core loaded into the receptor medium.

Many previous studies have investigated the efficacy and safety of minoxidil for the treatment of AGA. In 1986, a double-blind, placebo-controlled trial of minoxidil in extensive AGA showed excellent hair regrowth in 27.3% of subjects treated with minoxidil compared to 7.1% of the subjects in the control group.[18] Another study suggested a similar acceptable hair

Table 4: The secondary endpoints (adverse reactions) frequency in the intervention and control groups

Adverse reactions	Intervention group (n = 35)	Control group (n = 35)
Erythema	5 (14.28%)	1 (2.85%)
Burning	6 (17.14%)	1 (2.85%)
Itching	6 (17.14%)	3 (8.57%)
Papule	0	0
Urticaria	1 (2.85%)	0
Folliculitis	1 (2.85%)	1 (2.85%)
Total	19	6

regrowth in both 2% and 3% solutions of minoxidil compared to the placebo group.[19] Olsen *et al.*[20] investigated the efficacy of topically applied 2% and 5% solutions of minoxidil on male pattern AGA in a randomized clinical trial. It was reported that although both 2% and 5% solutions of minoxidil were effective against AGA, the 5% solution showed an earlier onset of therapeutic effects and showed 45% more hair regrowth than 2% topical minoxidil solution after 48 weeks of treatment. In a similar study, the effects of 2% and 5% solutions of minoxidil were investigated on a total of 381 women, indicating the superiority of minoxidil solutions, especially the 5% solution to the placebo group after 48 weeks of treatment.[21] In 2007, Olsen *et al.*[17] evaluated the effects of a novel foam formulation on AGA in men. It was reported that the foam formulation was indicated a significant increase ($P < 0.0001$) in hair counts

compared to the placebo group. Hillmann *et al.*[16] in 2015 conducted a randomized, placebo-controlled clinical trial to evaluate the efficacy of topical minoxidil foam on the vertex and frontotemporal AGA. It was investigated that the topical minoxidil foam caused a significant improvement in total coverage of scalp on both frontotemporal (P = 0.016) and vertex (P = 0.027) areas.

In the present study, it was observed that the aqueous-based nanosuspension formulation caused an enhanced hair diameter after 24 weeks of application (2.22 ± 3.00 µm). The marketed product showed an average of 1.31 ± 2.66-µm increase in hair diameter. The total hair count changes at baseline in the intervention and control groups were found to be 11.02 ± 7.76 and 9.82 ± 6.81 hairs/cm^2, respectively. The results suggested that although both products are effective on hair thickening and enhancing hair density, there were no statistically significant differences between the intervention and control groups for the primary endpoints (P > 0.05). The investigators suggested that a slightly higher number of subjects (42.85%) showed improvement in the total coverage of scalp in comparison to the control group (34.28%). Figure 5 illustrated the scalp coverage changes in two patients who received the nanosuspension of minoxidil. Because of the absence of ethanol in the formulation of aqueous-based nanosuspension of minoxidil, fewer complaints of adverse reactions were recorded in the intervention group compared to the control group. The number of reported complaints of adverse reactions was higher in the control group than in the intervention group, that is, erythema (14.28% against 2.85%), burning (17.14% against 2.85%), itching (17.14% against 8.57%), and urticaria (2.85% against none).

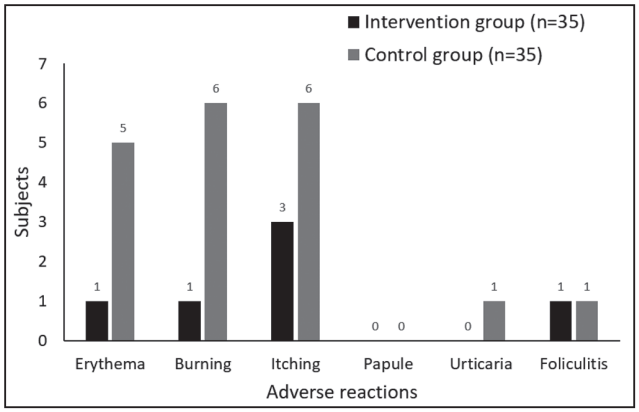

Figure 4: Comparison of the number of complaints about different adverse reactions (erythema, burning, itching, papule, urticaria, and folliculitis), reported by intervention and control groups

Conclusions

In this study, the clinical effect of an aqueous-based topical 2% nanosuspension of minoxidil was compared to that of the commercially manufactured topical solution of the drug on hair

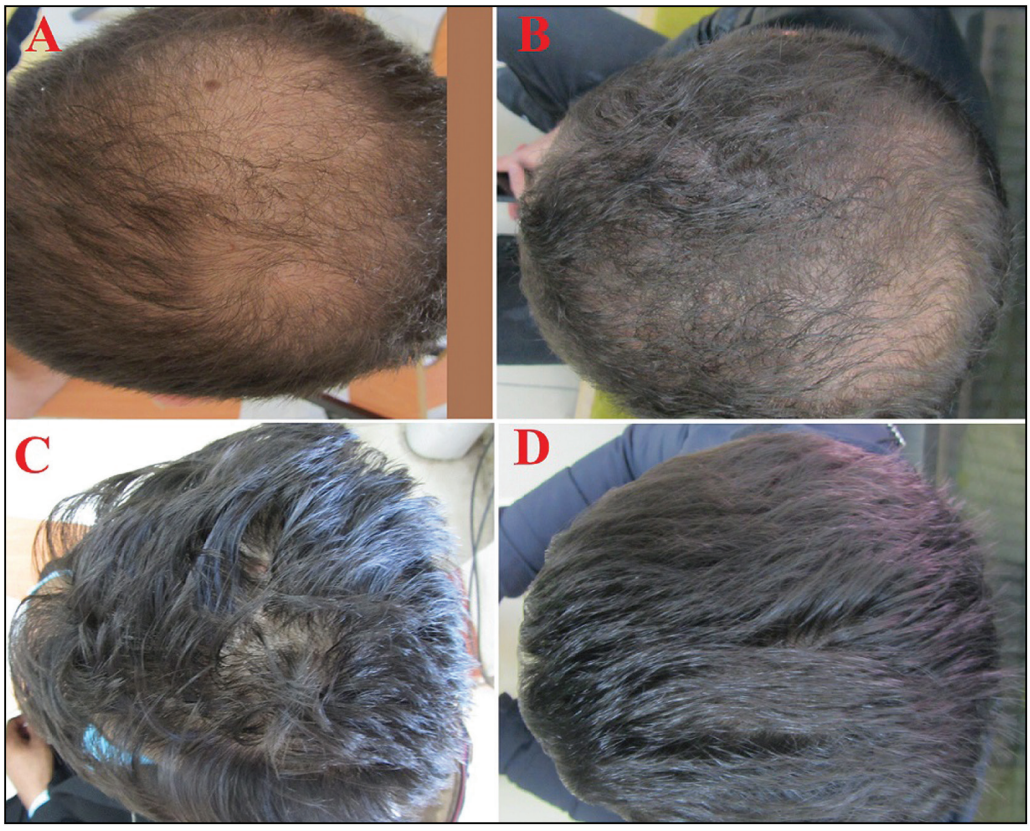

Figure 5: The overall changes in scalp coverage in two subjects treated with the aqueous-based topical 2% nanosuspension of minoxidil. (a) Subject #1 before treatment; (b) subject #1 after treatment; (c) subject #2 before treatment; (d) subject #2 after treatment

regrowth. Suitable physicochemical properties including almost 200-nm particle size, appropriate zeta potential distribution, uniform morphology, and two-step sustained *in vitro* drug release were detected for the nanosuspension formulation. Based on the clinical findings, this formulation was found to be effective in the treatment of AGA areata in both males and females, same as the marketed product. No statistically significant difference in the hair density and thickness changes at baseline was found between the groups treated with either nanosuspension or conventional solution. Furthermore, the aqueous-based topical 2% nanosuspension formulation showed better patient compliance, safety, and tolerability compared to the marketed product containing ethanol. Hence, it can be concluded that the aqueous-based topical 2% nanosuspension of minoxidil is suitable as a replacement for the conventional formulation.

Patient consent statement

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Organization: Kermanshah University of Medical Sciences
Place: Kermanshah, Iran

Conflicts of interest

There are no conflicts of interest.

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