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Evaluation of Topical Sodium Valproate-Loaded Nano Spanlastics Formulation versus Minoxidil® 5% in The Treatment of Female Pattern Hair Loss Using Transdermal Delivery: A Comparative Study

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ABSTRACT

Background: Valproic acid (VPA), is often used as an anticonvulsant drug that activates the Wnt/β-catenin signaling pathway and blocks GSK3b, a glycogen synthase kinase, and though connected with increasing hair growth and induction of anagen. Currently, only FDA-approved two percent topical minoxidil is available therapy to treat female pattern baldness. **Objective:** This randomized controlled clinical trial study aimed to evaluate the effectiveness of topical VPA and 5% topical minoxidil for treating Female pattern hair loss (FPHL).

Patients and methods: This study included 81 patients with FPHL who were allocated into three groups randomly (27 treated with topical sodium valproate-loaded nano spanlastics formulation, 27 treated with minoxidil® 5% lotion, and 27 treated with saline bottled containers using transdermal delivery using a derma roller for 6 months. The included patients were chosen randomly from the Outpatient Clinic of Dermatology, Andrology & STDs Department, Mansoura University Hospitals. All patients were subjected to history taking, and general dermatological and scalp examination.

Results: In comparison to the control group, the mean change in the total number of hairs was substantially larger in the VPA and minoxidil groups (P = 0.047). Although the majority of adverse events in both groups were moderate and self-limiting, the variations in prevalence rates across the groups were comparable (P = 0.72).

Conclusion: Topical VPA and 5% minoxidil increased the total hair count of our patients ; therefore, topical VPA is a potential therapy option for FPHL.

Keywords: Female Pattern Hair Loss, β-catenin, Glycogen synthase kinase 3, Hair, Valproic acid, Wnt, Minoxidil.

INTRODUCTION

The gradual transformation of thick, pigmented terminal hair into short, thin, non-pigmented vellus hair is the main character of female pattern hair loss (FPHL), non-scarring alopecia. Hair follicle miniaturization is the name of this undesirable process[(1)].

Much recent research examined the connection between androgenetic alopecia (AGA) and Wnt/ catenin signaling, a series of signal transduction pathways that start with proteins that transfer signals into the cell through cell surface receptors [(2)]. Valproic acid has recently been shown to be more efficient than Minoxidil at stimulating the renewal of hair follicles [(3,4)].

The carboxylic acid derivative (VPA) activates some signaling pathways, including, Wnt/catenin signaling which is implicated in hair formation [(5)]. According to some studies, By inhibiting glycogen synthase kinase 3b (GSK3b), VPA promotes hair follicles' move from the telogen (resting phase) to the anagen (active phase) [(6)]. Furthermore, Wnt elevates catenin levels significantly, but when it is not present, GSK 3b phosphorylates and degrades -catenin proteins. Therefore, We believe that when VPA is applied, it causes (i) Wnt protein to bind to cell surface receptors, (ii) GSK-3b to be prevented from phosphorylating -catenin, and (iii) the proliferation of Hair Follicle stem cells [(7)]. Topical administration of solvent-based VPA is the only method of administration that can stimulate the development of new hair [(8)]. Topical VPA administration that was accurate and effective was previously unfeasible, but to get around these limitations, dissolvable microneedles (DMNs) were developed to move large molecules over the epidermal barrier [(9)].Therapeutics can be encapsulated in DMNs and released minimally invasively upon skin implantation [(10)]. In human dermal papilla cells, minoxidil® prolongs the anagen phase and stimulates the β-catenin transcriptional pathway. These small results could be enough to halt follicular shrinking or promote hair growth in balding scalps [(11)]. This study aimed to evaluate the effectiveness of topical VPA and 5% topical minoxidil for treating Female pattern hair loss (FPHL).

PATIENTS AND METHODS

Study population

Female patients, 19–45 years old who had FPHL graded according to Ebling and Rook classification were recruited from the Outpatient Clinic of Dermatology, Andrology, STDs Department, Mansoura University Hospitals. Patients with abnormal physical or laboratory results, systemic illness such as heart and renal diseases, a hair transplantation history, 3 months topical minoxidil treatment, or any drugs such as dutasteride and finasteride for 6 months and pregnant or lactating females were excluded.

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Ethical approval: This study was approved by the institutional review board (IRB) of the Mansoura Faculty of Medicine (no. H-1003-067-313). Every patient who took part in this trial gave written, informed consent. This study was conducted in compliance with the code of ethics of the world medical association (Declaration of Helsinki) for human subjects.

Study agent: A colorless tonic lotion containing Sodium valproate, Poloxamers (P407, P188), κ-carrageenan, sodium chloride, Tween 80, Span 60and ethanol. The preparation of the spanlastic vesicles was done using the ethanol injection approach.). Span 60 and valproic acid was dissolved in ethanol (10 ml). The aqueous phase included appropriate edge activators (10 ml) (Tween 80). The warmed aqueous phase was receiving an injection of the organic phase, which was then be continuously agitated at 320 rpm on a magnetic stirrer to maintain a temperature of 60°C.

Heat was used to evaporate the solvent, leaving behind drug-filled spanlastic vesicles. The resulting vesicles were at ambient temperature, resulting in a uniform, particle-free, milky white suspension of spanlastics. Once a stable suspension of spanlastics has been created, sonication was applied to it [(12)].

Sodium valproate-loaded nano spanlastics formulation was applied twice daily Vs 5% minoxidil® lotion applied once daily using dermaroller in every application for 6 months. We used the marketed 5% minoxidil® mini pharmaceutical solution [prescribed an application of 1–2 ml of 5% minoxidil® solution once daily using dermaroller]. The placebo was a colorless tonic saline bottled container daily for 6 months using the same transdermal delivery.

Study design

It was a randomized controlled clinical trial for this pil ot study. (phaseIII). Randomization type: Simple Blinding (masking) was applied to the present study. Triple-blinded study (blinding of patients, treating physicians, and study assessors). No justification was needed. A sample size of 27 in each group was needed. For possible attrition rate, the total sample size was increased to 90.

Study groups:

Sodium Valproate group: 27 female patients treated with topical sodium valproate-loaded nano spanlastics formulation using transdermal delivery using derma roller to stimulate the synthesis of endothelial growth factors to promote hair growth in follicles, we used (1 to 1.5mm) derma rollers according to the case twice daily for 6 months.

Minoxidil® group: 27 female patients treated with minoxidil® 5% lotion applied once daily for 6 months using the same trans-dermal delivery.

Control group: 27 female patients treated with saline bottled containers daily for 6 months using the same trans-dermal delivery. Six months of therapy in all.

Compliance evaluation: We gave the test subjects a new lotion of sodium valproate-loaded nano spanlastics formulation (150 mL) at baseline, and 1 to 6 months which is the treatment period.

Efficacy assessment: A trichoscopy study was carried out as previously described to evaluate the topical VPA's efficacy objectively Canfield Scientific's camera system was employed. to take 4 pictures of a 1cm^2 circular scalp area 2 cm apart frome the vertex (Fairfield, NJ, USA).

Before employing Automated Digital Image Analysis Software, the image was submitted to image analysis. A computer imaging system was used to quantify hair counts, the study's main endpoint, at baseline and six months following treatment. Hair growth rate and diameter were the secondary endpoints, and they were also assessed at baseline and six months after the treatment. We took three-day interval images, to calculate the rate of hair growth, each hair was matched, and the difference in hair length was evaluated.

Safety assessment

The patients were assessed in the clinic at 2, 4, and 6 months for safety using hair analysis and patient documentation of side effects. The intensity of side effects was divided into four categories: mild (no daily affection), moderate (minimal daily affection), severe (hospitalization required), and life-threatening. The IRB kept track of any serious or fatal side effects that occurred.

Statistical analysis

Statistical Package for Social Science (IBM Corp., 2017) was used to gather, review, code, tabulate, and introduce the data to a computer. Armonk, New York: IBM Corp., IBM SPSS Statistics for Windows, Version 25.0. Quantitative data were reported as mean+SD or median (range) following normalcy, whilst qualitative data was given as numbers and percentages. According to the kind of data gathered for each parameter, the appropriate analysis was carried out, such as an ANOVA for comparing more than two groups, means with post hoc testing, and a Chi-Square test to look at the correlation between two qualitative variables. To compare means across one or more variables based on repeated observations, use the Repeated Measures ANOVA method. A was regarded as statistically significant if the P value was less than 0.05.

RESULTS

A total of 81 female patients AGA were taken from late October 2021 till April 2022 and divided into 3 groups: VPA group (n = 27), Minoxidil group (n= 27), and control group (n = 27).

Demographic characteristics

Table 1 displays the demographic data, which revealed no statistically significant differences between the three groups. As regard age and BMI. The

mean (age ± SD) of the Sodium valproic group, Minoxidil group & Control group was 30.45 ± 7.62, 32.05 ± 6.72 & 30.87 ± 6.97 years, respectively. The mean (BMI ± SD) of the Sodium valproic group, Minoxidil group & Control group was 29.69 ± 5.40, 31.57 ± 5.65 & 29.66 ± 4.21 Kg/m2.

Table (1): Comparison of the demographic data in the three study groups

Variable	Sodium valproic group (N=27)	Minoxidil group (N= 27)	Control group (N= 27)	Test of sig.
Age (years)	30.45 ± 7.62	32.05 ± 6.72	30.87 ± 6.97	F= 1.335, P = 0.188
BMI (Kg/m^2)	29.69 ± 5.40	31.57 ± 5.65	29.66 ± 4.21	F= 1.663, P = 0.146

F= One-way ANOVA, P= Intergroup significance

According to Ebling and Rook categorization, there was no statistically significant difference between the three groups (Sodium valproic group, Minoxidil group, and Control group) in terms of illness duration, family history, or AGA grade (P = 0.118, P = 0.104, and P = 0.104, respectively) (2). The mean disease (duration ± SD) of the Sodium valproic group, Minoxidil group, and the control group was 4 (1-8) years, 4 (1-9) years & 3 (1-7) years, respectively. Family history was positive in 44.4%, 37% &48.2% of the Sodium valproic group, Minoxidil group & Control group, respectively. Grade 1(33.3%) and 2 (33.3%) were the most common AGA grades among Sodium valproic group while grade 2 was the most common grade among both the Minoxidil group (44.4%) and control group (40.7%).

Table (2): Comparison of the clinical data in the three study groups

Variable	Sodium valproic group (N=27)	Minoxidil group (N= 27)	Control group (N= 27)	Test of sig.
Disease duration (years)	**4 (1-8)**	**4 (1-9)**	**3 (1-7)**	**KW= 1.926 P = 0.118**
Family history				
Negative	**15 (55.6%)**	**18 (63%)**	**14 (51.8%)**	**χ2 = 2.164 P = 0.104**
Positive	**12 (44.4%)**	**10 (37%)**	**13 (48.2%)**	
Ebling and Rook classification				
Grade 1	**9 (33.3%)**	**7 (25.9%)**	**10 (37%)**	**MC = 2.164 P = 0.104**
Grade 2	**9 (33.3%)**	**12 (44.4%)**	**11 (40.7%)**	
Grade 3	**5 (18.5%)**	**4(14.8%)**	**3 (11.1%)**	
Grade 4	**4(14.8%)**	**4(14.8%)**	**3 (11.1%)**	

KW= Kruskal Wallis test, χ2=Chi-square test MC: Mone-Carlo test, P= Intergroup significance.

Efficacy assessment

According to **Table (3),** the mean hair follicle (density SD) before treatment was 158.46± 23.1 /cm^2 in the valproic acid group, 156.17± 20.94 /cm^2 in the minoxidil group, and 157.65 21.48 /cm^2 in the control group (there was no obvious difference between the three groups. The average number of hair follicles (density SD) in the valproic acid group was 162.97± 25.45/cm^2, in the minoxidil group it was 153.2±19.17/cm^2, and in the control group, it was 154.30±19.72/cm^2 after one month. This represents a statistically significant increase in hair follicle density in the valproic acid group in comparison to the minoxidil group and control group (P=0.017*).

In the valproic acid group, the mean hair follicle density (density SD) was 168.16±27.10/cm^2, 159.33±20.27/cm^2 in the minoxidil group, and 156.39±18.45/cm^2 in the control group after two months of therapy. This increase in average hair follicle density in the valproic acid group in comparison to both the minoxidil group and the control group is statistically significant (P=0.005*).

After three months of treatment, the average number of hair follicles (density SD) in the valproic acid group was 174.09±30.76/cm^2, in the minoxidil group it was 172.15±27.34/cm^2, and in the control group, it was 158.24±17.05/cm^2. There was a statistically significant increase in hair follicle density in both the valproic acid group and the minoxidil group.

The average number of hair follicles (density SD) in the valproic acid group was 81.47±35.19/cm^2, in the minoxidil group it was 179.64±31.52/cm^2, and in the control group, it was 155.96±17.22/cm^2 after four months. There was a statistically significant increase in hair follicle density in both the valproic acid group and the minoxidil group in comparison to the control group.

After five months of therapy, the average number of hair follicles (density SD) in the valproic acid group was 188.16±36.09 /cm^2, in the minoxidil group it was 183.51±33.08 /cm^2, and in the control group, it was 154.77±13.72 /cm^2. There was a statistically significant increase in hair follicle density in both the valproic acid group and the minoxidil group.

After six months of therapy, the mean hair follicle (density SD) in the valproic acid group was 194.57±43.01 /cm^2, 187.16±32.84 /cm^2 in the

minoxidil group, and 157.64±21.48 /cm^2 in both the valproic acid group and the minoxidil group experienced statistically significant increases in mean hair follicle density when compared to the control group. After six months of therapy, both the sodium valproic group and the minoxidil group had significantly higher mean hair follicle densities than they had before treatment.

Table (3): Comparison of hair density (/cm2) in the three study groups

Variable	Sodium valproic group (N=27)	Minoxidil group (N= 27)	Control group (N= 27)	Test of sig.
Pretreatment	158.46 ± 23.1 A	156.17 ± 20.94 A	157.65 ± 21.48 A	F= 1.628 P= 0.354
1 month after treatment	162.97 ± 25.45 A	153.2 ± 19.17 B	154.30 ±19.72 B	F= 3.244 P= 0.017*
2 months after treatment	168.16 ± 27.10 A	159.33 ± 20.27 B	156.39 ± 18.45 B	F= 4.218 P= 0.005*
3 months after treatment	174.09 ± 30.76 A	172.15 ± 27.34 A	158.24 ± 17.05 B	F= 4.805 P= 0.001*
4 months after treatment	181.47 ± 35.19 A	179.64 ± 31.52 A	155.96 ± 17.22 B	F= 5.334 P < 0.001*
5 months after treatment	188.16 ± 36.09 A	183.51 ± 33.08 A	154.77 ± 13.72 B	F= 6.125 P < 0.001*
6 months after treatment	194.57 ± 43.01 A	187.16 ± 32.84 A	157.64 ± 21.48 B	F= 7.340 P < 0.001*
Mean percent of change (%)	23.24	19.81	1.9	
Repeated measures ANOVA	P1 < 0.001*	P1 < 0.001*	P1= 0.848	

F= One-way ANOVA *: Statistically significant ($p \leq 0.05$)
P= Intergroup significance
P1: Comparison between basal and final value in each group (Value of repeated measures ANOVA)
A, B, C: Similar results indicate no significant difference between adjacent groups, different letters indicate a statistically significant difference between adjacent groups

At baseline and after 1, 2, 3, 4, 5, and 6 months of therapy, photographs are displayed **(Fig. 1).**

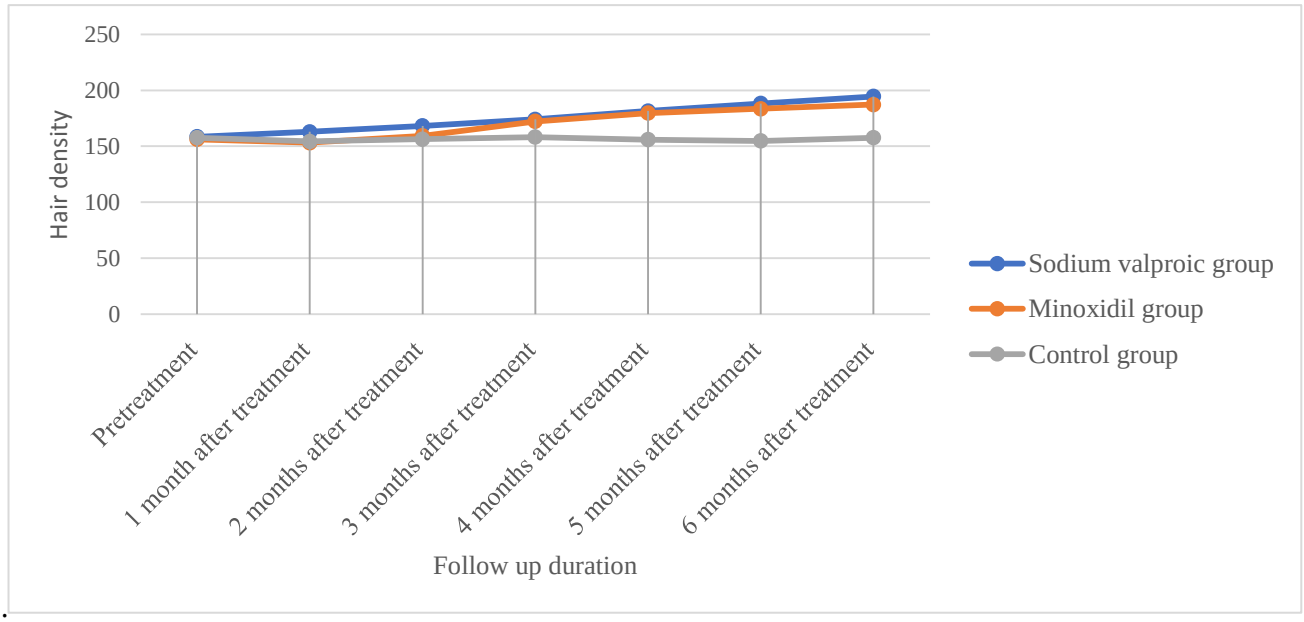

Figure (1): The hair follicle density with time in the instances that were examined.

There was a significant statistically high rise in the density of hair follicles after 6 months of treatment in comparison with pretreatment in both the sodium valproic group and minoxidil groups (p< 0.001*) as in **figure (2).**

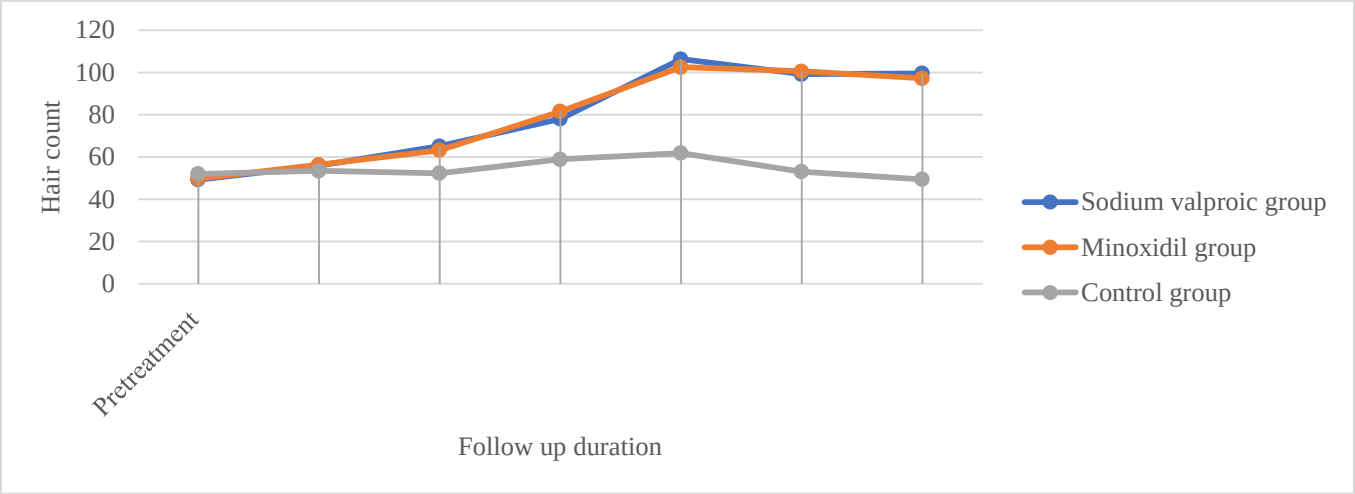

Figure (h2): The cases included in the study's hair count follow-up.

Before treatment, the mean hair (count SD) in the valproic acid group was 49.26±6.69 /cm2, in the minoxidil group it was 49.74±9.33 /cm2, and in the control group, it was 51.99±11.52 /cm2, with no obvious difference between the three groups (p=0.360).

The mean hair (count SD) in the valproic acid group was 55.95±12.39/cm2, in the minoxidil group it was 56.37±11.58/cm2, and in the control group, it was 53.46 10.56/cm2, with no obvious differences between the three groups (p=0. 232).

After two months of therapy, the mean hair (count SD) in the valproic acid group was 65.1±13.71 /cm2, 63.21±17.61 /cm2 in the minoxidil group, and 52.32 8.04 /cm2 in the control group. Both the valproic acid group and the minoxidil group experienced statistically significant increases in mean hair counts when compared to the control group (P=0.013*).

After 4 months of therapy, the mean hair (count SD) in the valproic acid group was 106.29±22.35/cm2 after three months of therapy, the mean hair (count SD) in the valproic acid group was 78.06±19.29 /cm2, 81.57±20.1 /cm2 in the minoxidil group, and 58.92±10.26 /cm2 in the control group. Both the valproic acid group and the minoxidil group experienced statistically significant increases in mean hair count when compared to the control group (P=0.002*). 102.54±21.03 /cm2 in the minoxidil group, and 61.83±10.59 /cm2 in the control group. Both the valproic acid group and the minoxidil group experienced statistically significant increases in mean hair count when compared to the control group (P 0.001).

The mean hair (count SD) in the valproic acid group was 99.15±22.23 /cm2, the mean hair (count SD) in the minoxidil group was 100.56±24.45 /cm2, and the mean hair (count SD) in the control group was 53.1±12.84 /cm2. There was a statistically significant increase in the mean hair count in both the valproic acid group and the minoxidil after six months of therapy, the mean hair (count SD) in the valproic acid group was 99.54±20.13 /cm2, 97.23±21.66 /cm2 in the minoxidil group, and 49.44±8.82 /cm2 in the control group. Both the valproic acid group and the minoxidil group experienced statistically significant increases in mean hair counts when compared to the control group (P= 0.001*).

Table (4): Comparison of hair count in the three study groups

Variable	Sodium valproic group (N=27)	Minoxidil group (N= 27)	Control group (N= 27)	Test of sig.
Pretreatment	49.26 ± 6.69 A	49.74 ± 9.33 A	51.99 ± 11.52 A	F= 1.318, P= 0.360
1 month after treatment	55.95 ± 12.39 A	56.37 ± 11.58 A	53.46 ± 10.56 A	F= 1.842, P= 0.232
2 months after treatment	65.1 ± 13.71 A	63.21 ± 17.61 A	52.32 ± 8.04 B	F= 3.942, P= 0.013*
3 months after treatment	78.06 ± 19.29 A	81.57 ± 20.1 A	58.92 ± 10.26 B	F= 4.896, P= 0.002*
4 months after treatment	106.29 ± 22.35 A	102.54 ± 21.03 A	61.83 ± 10.59 B	F= 6.114, P< 0.001*
5 months after treatment	99.15 ± 22.23 A	100.56 ± 24.45 A	53.1 ± 12.84 B	F= 5.735, P< 0.001*
6 months after treatment	99.54 ± 20.13 A	97.23 ± 21.66 A	49.44 ± 8.82 B	F= 5.907, P< 0.001*
Mean percent of change (%)	102.61	97.9	19.6	
Repeated measures ANOVA	P1 < 0.001*	P1 < 0.001*	P1= 0.322	

F= One-way ANOVA *: Statistically significant (p ≤ 0.05), P= Intergroup significance,
P1: Comparison between basal and final value in each group (Value of repeated measures ANOVA)

Similar findings show no statistically significant difference between neighboring groups, but different letters show a difference that is statistically significant between adjacent groups (A, B, and C).

The mean hair count increased significantly, as measured by statistics, after 6 months of treatment in comparison with pretreatment in both the sodium valproic group and minoxidil groups (p< 0.001*) as in **figure (3).**

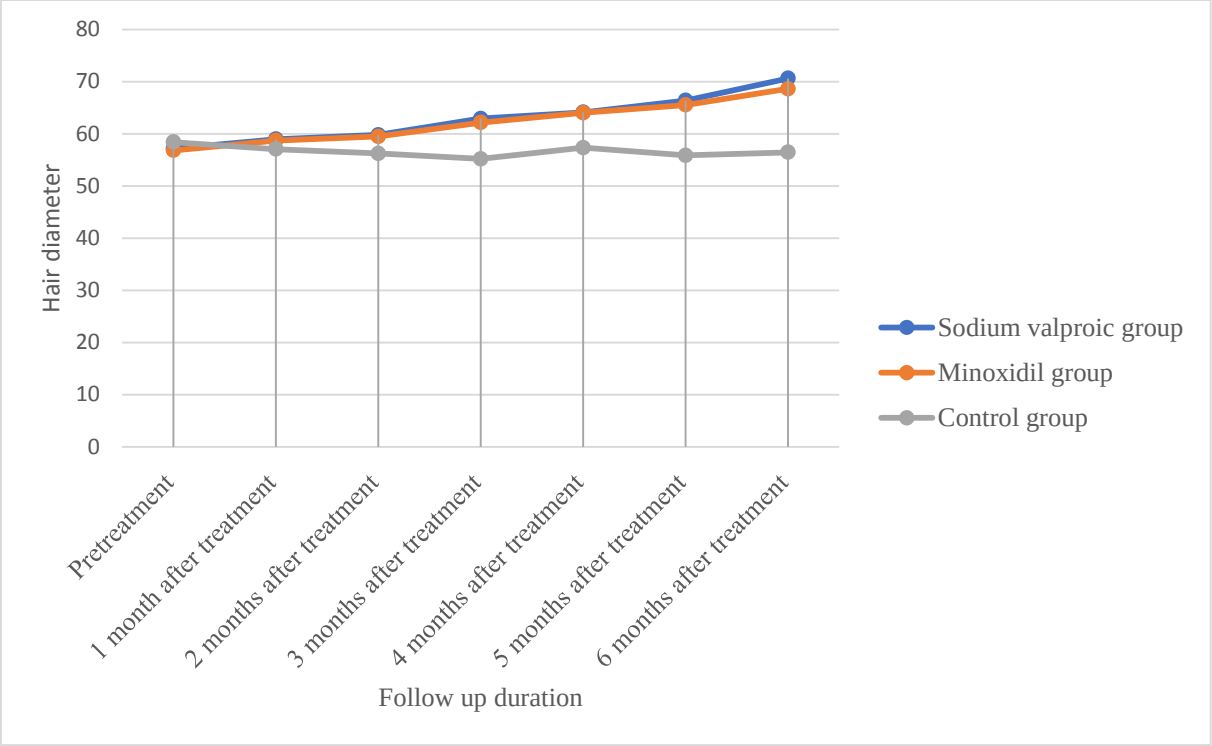

Figure (3): Follow-up of the hair shaft diameter in the studied cases.

Before treatment, the mean diameter of the hair shafts in the valproic acid group was 57.18±11.54 µm, in the minoxidil group they were 56.8±10.3 µm, and in the control group, they were 58.4±13.2 µm (p=0.336).

The valproic acid group's mean hair shaft diameter was 58.94±13.15 µm after a month of therapy.

After three months of treatment, the mean diameter of hair shafts in the valproic acid group was 62.92± 15.65 µm, in the minoxidil group it was 62.15±15.33 µm, and in the control group, it was 55.17±11.43 µm. Both the valproic acid group and the minoxidil group experienced a statistically significant increase in mean hair diameter when compared to the control group (p=0.032*). Compared to the control group, both the valproic acid and minoxidil groups had a statistically significant increase in the mean hair diameter (p=0.005*).

After six months of treatment, the mean diameter of hair shafts in the valproic acid group was 70.60±23.46 µm, in the minoxidil group it was 68.62±17.13 µm, and in the control group, it was 56.45±12.48 µm. Both the valproic acid group and the minoxidil group experienced a statistically significant increase in mean hair shaft diameter when compared to the control group (p=0.001*).

Table (5): Comparison of hair diameter (µm) in the three study groups

Variable	Sodium valproic group (N=27)	Minoxidil group (N= 27)	Control group (N= 27)	Test of sig.
Pretreatment	57.18 ± 11.54 A	56.8 ± 10.3 A	58.4 ± 13.2 A	F= 1.350, P= 0.336
1 month after treatment	58.94 ± 13.15 A	58.71 ± 11.24 A	57.04 ± 12.69 A	F= 1.127, P= 0.484
2 months after treatment	59.77 ± 14.39 A	59.48 ± 13.91 A	56.23 ± 13.32 A	F= 1.892 P= 0.134
3 months after treatment	62.92 ± 15.65 A	62.15 ± 15.33 A	55.17 ± 11.43 B	F= 3.067 P= 0.032*
4 months after treatment	64.12 ± 16.01 A	64.02 ± 15.87 A	57.34 ± 13.22 B	F= 3.406 P= 0.019*
5 months after treatment	66.38 ± 16.41 A	65.51 ± 15.83 A	55.87 ± 13.80 B	F= 3.927, P= 0.005*
6 months after treatment	70.60 ± 17.46 A	68.62 ± 17.13 A	56.45 ± 12.48 B	F= 4.319 P= 0.001*
Mean percent of change (%)	22.7	20.8	5.1	
Repeated measures ANOVA	0.001*	0.001*	0.344	

F= One-way ANOVA *: Statistically significant ($p \leq 0.05$), P= Intergroup significance

P1: Comparison between basal and final value in each group (Value of repeated measures ANOVA)

A, B, C: Similar results indicate Different letters denote statistically significant differences between neighboring groups when there is no difference between them.

In both the sodium valproic group and the minoxidil group, the mean hair shaft diameter statistically substantially increased after 6 months of therapy in comparison to pretreatment (p 0.001*), as shown in figure (4).

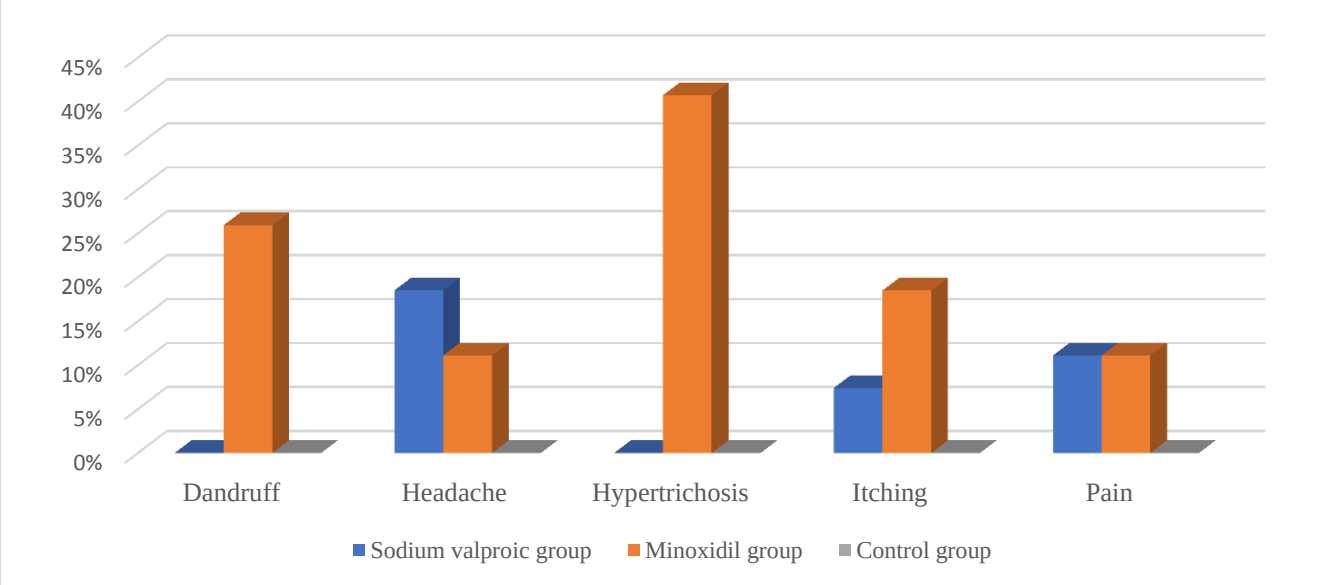

Figure (4): Analysis of the adverse effects in the study's cases.

Safety assessment

Regarding the associated side effects, 7 cases (25.9%) suffered from dandruff in minoxidil statistically, the treated group differed from the other two groups (P=0.025*). In the minoxidil-treated group, there were 11 cases (40.7%) of face hypertrichosis, with a statistically significant difference between the three groups (p=0.128).

There was no statistically significant difference in the occurrence of discomfort, itching, or headache between the three groups (P>0.05 for each). Regarding the concomitant side effects, as indicated in Table (6), there was a statistically significant difference between the three groups (P=0.025*) in that 7 instances (25.9%) of the minoxidil group had dandruff.

In the minoxidil group, there were 11 cases (40.7%) of reported face hypertrichosis, with a statistically significant difference between the three groups (p=0.128). There was no statistically significant difference in the occurrence of discomfort, itching, or headache between the three groups (P>0.05 for each).

Table (6): Comparison of the side effects in the three study groups

Variable	Sodium valproic group (N=27)	Minoxidil group (N= 27)	Control group (N= 27)	Test of sig.
Dandruff	0 (0%)	7 (25.9%)	0 (0%)	MC= 3.514 P= 0.025*
Headache	5 (18.5%)	3 (11.1%)	0 (0%)	MC= 2.531 P= 0.246
Hypertrichosis	0 (0%)	11 (40.7%)	0 (0%)	MC= 4.152 P= 0.005*
Itching	2 (7.4%)	5 (18.5%)	0 (0%)	MC= 2.360 P= 0.285
Pain	3 (11.1%)	3 (11.1%)	0 (0%)	MC= 1.956 P= 0.325

MC: Mone-Carlo test, *: Statistically significant (p≤ 0.05), P= Intergroup significance.

DISCUSSION

For FPHLTopical minoxidil is advised as the initial course of treatment. A potassium channel opener called minoxidil is thought to increase angiogenesis, anti-inflammatory, and antiandrogenic characteristics, and cause vasodilation [13].

An anticonvulsant medicine called valproic acid (VPA) was initially investigated for its ability to promote hair growth when applied topically by **Lee *et al.*** [4] in 2012, utilizing male mouse mice. Topical administration of solvent-based VPA is the only method of administration that can stimulate the development of new hair (topical VPA). The solvent is utilized to improve chemicals to aid VPA's passage across the epidermal barrier [14].

VPA inhibits several signaling pathways, such as the Wnt/b-catenin, extracellular signal-regulated kinase, and protein kinase C, which in turn affects gene expression [15, 16].

Topical VPA treatment significantly boosted hair growth in a mouse trial, whereas oral VPA administration had no such effect [4]. As a result, to prevent any possible systemic effects of VPA on the activity of biotinidase or carboxylase, we employed VPA as a topical treatment in this investigation.

VPA is easily absorbed via the skin due to its low molecular weight of 144.2 g/mol. Additionally, it is stable at ambient temperature and readily soluble in water [8].

After 6 months of therapy, the average hair follicle density considerably increased statistically when compared to pretreatment in both the sodium valproic group and the minoxidil group (p 0.001*).

To the best of our knowledge, this study is the first reported study that compares the efficacy of topical 8.7% sodium valproate-loaded nano spanlastics formulation to minoxidil® 5% in treating FPHL using a transdermal administration technique. According to **Badria *et al.*** [17], who assessed the effectiveness of Valproic acid (group A) Versus Minoxidil (group B) as Topical Treatment for Male and Female Androgenetic Alopecia, this result was consistent with their findings.

The effects of topical VPA on androgenetic alopecia were investigated experimentally in South Korea using murine models and human dermal papilla cells. VPA was regularly administered topically to the backs of C3H mice in various amounts .

VPA-treated mice's hair follicles show hair growth and reached the anagen phase, whereas control mice did not. The telogen phase persisted in the vehicle solution-treated control group.

According to the histomorphometric assessments, VPA enhanced the telogen-anagen transition. VPA boosted the filaggrin and loricrin expression, according to an immunohistochemical investigation [4].

Jo *et al.* [8] conducted a clinical trial that is randomized, double-blind, and controlled for placebo effectiveness of topical VPA for the treatment of AGA. For 24 weeks, male patients with moderate AGA received either VPA (sodium valproate 8.3%) or a placebo spray as therapy. The change in hair count during therapy, as determined by photo-trichogramma analysis, served as the main outcome for effectiveness. Age, length of hair loss, and baseline total hair count did not differ statistically across the groups.

In comparison to the placebo group. the VPA group's mean change in total hair count increased significantly. To treat AGA, **Choi *et al.*** [18] looked into the possibility that VA could have an impact on the hair cycle. The unknown mechanism is behind VPA's effects on the hair. The chelating of metals and inhibition of metallic enzymes, which are crucial for keratinization and hair development, are the proposed mechanisms [19].

In the current study, both the sodium valproic group and the minoxidil group had an increase in mean hair count that was statistically significant compared to pretreatment following a 6-month therapeutic period (p 0.001*).

These results supported those of **Badria *et al.* [17],** who discovered that there was no change in the mean number of hair follicles before treatment between groups A and B ($p > 0.05$), which was 17.9± 2.1/cm^2 for group A and 18.3±2.75 /cm^2 for group B, respectively. In group B, there was a more noticeable initial decline in the number of hair follicles in the first and second months, and there was a statistically significant difference (p 0.05) between the two groups .

That might be explained by early telogen hairs falling out as a result of resting hair follicles prematurely transitioning to the anagen phase of hair development [20].

The mean number of hair follicles increased significantly (high statistical significance) in groups A and B after six months of therapy, reaching 31.58±6.49/cm^2 and 32.54±6.39/cm^2, respectively [17].

In the current study, both the sodium valproic group and the minoxidil group show statistically significant increases in the mean hair shaft diameter when compared to pretreatment at the end of six months of therapy (p 0.001). Similar to this, **Badria *et al.* [17]** found no appreciable change between group A and group B's mean hair shaft diameters of 51.5 ±5.27 µm and 50.7 ±5.44 µm, respectively, prior to treatment ($p > 0.05$).

The diameter of the hair shaft grew in both groups A and B after 6 months of therapy, reaching 62.4±7.54 µm and 63.19±7.49 µm, pre-and post-treatment in both groups, with a statistically significant difference between the two groups (p 0.001).

In terms of the concomitant side effects, the minoxidil-treated group experienced dandruff in 7 instances (25.9%), with a statistically significant difference between the three groups (P=0.025*).

There were 11 occurrences of reported facial hypertrichosis in the minoxidil-treated group (40.7%), with a statistically significant difference between the three groups (p=0.0128). There was no statistically significant difference in the occurrence of discomfort, itching, or headache between the three groups (P>0.05 for each).

According to research by **Badria *et al.* [17]**, group A experienced much less early shedding and discomfort than group B (p 0.001 and p 0.01, respectively). Furthermore, the participants in group B had 7.5% more incidences of facial hypertrichosis than group A individuals did, who had none. Groups A and B, where dandruff was present in 5% and 2.5% of instances, respectively, did not differ significantly from one another in this regard ($p > 0.05$). However, it was claimed that dandruff was present in the two cases in group A before they began their therapy, which included utilizing ketoconazole shampoo. These results demonstrate that VA is safer than minoxidil.

According to **Jo *et al.* [8],** 11 out of the 40 individuals experienced negative effects during the 24-week study. The majority of these side effects were mild and self-limited, and they included localized skin problems and sleeplessness. On the other hand, side symptoms associated with minoxidil usage have included temporary shedding during the first month of treatment, contact dermatitis, and face hypertrichosis. Without propylene glycol, the foam preparation is less prone to irritate [21].

Ghonemy *et al.* [22] indicated that all patients in group B (100%) had higher contact dermatitis due to drug-related irritation than group A (22.2%). Fewer erosions were seen in group A patients (11.1%) compared to six patients (25%) in group B. Compared to nine individuals in group B, only six patients in group A reported having hypertrichosis.

Only 11.1% of those who used minoxidil at 10% experienced headaches, compared to 25.0% of the minoxidil 10% group. The placebo group experienced zero adverse drug responses. In terms of therapeutic effectiveness, our comparative clinical research did not find any appreciable distinction between VPA and minoxidil lotion. After the first and second months of therapy, the VPA group saw a faster increase than the minoxidil group in terms of hair density.

Topical valproic also had fewer negative effects than minoxidil lotion and did not cause dandruff or face hypertrichosis. There were a few issues with the current study.

First, due to the tiny patient population, we advise future research to include a larger patient population from many centers. Additionally, larger concentrations may need to be looked for, and VPA spray dosages may be more beneficial for treating AGA because the ideal dose and concentration of the VPA formulation have not yet been established. Therefore, we advise routine follow-up to check for any potential hair loss after ceasing topical valproic acid medication and to suggest maintenance therapy if necessary. SV nanospanlastics may improve topical SV delivery for a therapy that is both safe and effective for FPHL, following the evidence.

CONCLUSION

Topical VPA and 5% minoxidil increased the total hair count of our patients; therefore, topical VPA is a potential therapy option for FPHL.

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