RESEARCH ARTICLE

CLINICAL TRIAL OF AN HERBAL PRODUCT IN THE TREATMENT OF ALOPECIA

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ARTICLE INFO

ABSTRACT

Background:
Androgenic Alopecia (AGA): Androgenic alopecia (also known as Androgenetic alopecia or Alopecia androgenetica), in male humans in particular this condition is also commonly known as male pattern baldness, hereditary alopecia and simply common baldness.

Pathophysiology: Androgenetic alopecia is a genetically determined disorder and is progressive through the gradual conversion of thick terminal hairs with fine, miniaturized hairs that are eventually lost. Patients with this disorder usually have a typical patterned distribution of hair loss.

In Androgenetic alopecia, Studies have indicated a self-renewal of the hair follicle via keratinocyte stem cells located at the area of the bulge of the hair follicle. In addition, a series of studies using mice has indicated that interfollicular keratinocyte stem cells could generate de novo hair follicles in adult mouse skin. These regenerated hair follicles cycled through stages of telogen to anagen. However, these transitions between bulge and epidermal keratinocytes have not been seen yet in human studies. Another report has indicated that mice lacking in functional vitamin D receptors develop a functional first coat of hair, but lack the cyclic regeneration of hair follicles leading to the development alopecia. Whether these findings will lead to a new area of exploration into the cause of Androgenetic alopecia in humans is unknown at this time.

Objectives:
Primary objective:
To evaluate the Efficacy of topical application of study drug for the treatment of mild to moderate Androgenic Alopecia in Male Patients.

Secondary objective:
To evaluate the safety of topical application of study drug for the treatment of mild to moderate Androgenic Alopecia in Male Patients.

Methods: Subjects were enrolled as per the inclusion and exclusion criteria. All the subjects were then randomized in to 5 different groups.
Drug A= FD/SBF/MX-40
Drug B- FD/SBF/PY-39
Drug C- FD/WBF/MX-36
Drug D- FD/WBF/PY-37
Drug E- FD/WBF/KX-38

INTRODUCTION

Androgenic Alopecia (AGA)

Androgenic alopecia (also known as Androgenetic alopecia or Alopecia androgenetica), in male humans in particular this condition is also commonly known as male pattern baldness, hereditary alopecia and simply common baldness.

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Pathophysiology

Androgenetic alopecia is a genetically determined disorder and is progressive through the gradual conversion of thick terminal hairs with fine, miniaturized hairs that are eventually lost. Patients with this disorder usually have a typical patterned distribution of hair loss. In Androgenetic alopecia, Studies have indicated a self-renewal of the hair follicle via keratinocyte stem cells located at the area of the bulge of the hair follicle. In addition, a series of studies using mice has indicated that interfollicular keratinocyte stem cells could generate de novo hair follicles in adult mouse skin. These

regenerated hair follicles cycled through stages of telogen to anagen. However, these transitions between bulge and epidermal keratinocytes have not been seen yet in human studies. Another report has indicated that mice lacking in functional vitamin D receptors develop a functional first coat of hair, but lack the cyclic regeneration of hair follicles leading to the development alopecia. Whether these findings will lead to a new area of exploration into the cause of Androgenetic alopecia in humans is unknown at this time.

Objectives

Primary objective: To evaluate the Efficacy of topical application of study drug for the treatment of mild to moderate Androgenic Alopecia in Male Patients.

Secondary objective: To evaluate the safety of topical application of study drug for the treatment of mild to moderate Androgenic Alopecia in Male Patients.

MATERIAL AND METHODS

The subjects were randomized in 5 different group and accordingly each group received only 1 particular study drug for tropical .Below are the list of drugs which were given to the subjects

Drug A= FD/SBF/MX-40
Drug B- FD/SBF/PY-39
Drug C- FD/WBF/MX-36
Drug D- FD/WBF/PY-37
Drug E- FD/WBF/KX-38

Study drug is Topical application 1 ml applied twice on the bald area of the scalp morning and evening (2 applications per day in total), up to 6 completed months.

Inclusion criteria

- Men aged 20 to 45 years, in general good health.
- Mild to moderate male-pattern baldness (androgenic alopecia), preferably on the top back of the skull.
- Willingness to maintain the same hair style, approximate length, and hair color throughout the study.
- Subject willing to continue his current regimen of vitamins and nutritional supplements and not start any new vitamins or nutritional supplements for the duration of the study.
- Able to read, understand, and provide written (signed) informed consent after the nature of the study has been fully explained and before any procedures dictated by this protocol were performed.
- Subjects must be willing and able to comply with follow-up requirements in a timely manner.

Exclusion criteria

- Any dermatological condition of the scalp other than androgenic alopecia (males).
- Prior use of scalp hair growth treatment (e.g., finasteride, minoxidil) within 6 months.
- Any prior hair growth procedures (e.g., hair transplant or laser).
- History of alcohol or drug addiction.
- History of skin allergy.

- Regular use of medication which might interfere with the results of the study.
- Subject had used phytotherapy (e.g., saw palmetto) within eight weeks prior to baseline.
- Any active skin infection in the scalp area or scarring in the target area.
- Photosensitivity to laser light.
- Subject had used Accutane® in the previous year. (Isotretinoin (sold as Accutane or roaccutane) Isotretinoin is an isomer of Vitamin A (13-cis-Vitamin An acid). Hair thinning or hair loss on the other hand, is one of the most commonly reported post accutane side effects.
- Medications with anti-androgenic properties (e.g., cyproterone acetate, spironolactone, ketoconazole, flutamide, bicalutamide), topical estrogen, tamoxifen, anabolic steroids, oral glucocorticoids (or other medications at the discretion of the Investigator. (These are the Medications for hair loss).
- History of thyroid or other medical condition that might influence hair growth and loss, at the discretion of the Investigator.
- Subject had "buzz" cut hairstyle, defined as hair cut to less than one inch in length.
- Subject had light blonde hair, at the discretion of the investigator.
- Subject had ever received radiation therapy to the scalp, or had chemotherapy within the past year.
- Subject had participated in any investigational study within the 30 days prior to randomization.
- A history or the presence of any serious and/or chronic medical condition(s) [including psychiatric illnesses] which, in the opinion of the investigator, may cause harm to the individual and/or compromise/confound the study results.
- Subject is currently enrolled in, or has not yet completed a period of at least 30 days since ending other investigational device or drug trial(s).
- Subjects unwilling or unable to comply with the study procedures.

Ethics Committee approval

All study related documents Protocol, CRF, Dairy Card, Investigator Brochure, SF – 36 and ICF (English and Kannada versions). Written informed consent was obtained from the subject(s) before the start of the trial and after due approval from IEC/IRB. Ethics Committee notifications as per the GCP guidelines issued by Central Drugs Standard Control Organization and ethical guidelines for biomedical research on human subjects issued by Indian Council of Medical Research has been followed during the conduct of the study. Two different Ethics committee were presented with the documents for the Ethics Committee Approval:

Dr. Venkat Charmalaya – EC Approval Date: 18-12-2011
Clinical Independent Ethics Committee-EC Approval Date: 24-03-12

Visit details

The patients were screened and enrolled. The enrollment day was considered as the baseline data and the patient were asked

to visit on: Day30 (Month 1), Day60 (Month 2), Day90 (Month 3), Day 120 (Month 4) and Day150 (Month 5).

Statistical analysis

All the continuous variables were is analyzed by using the Analysis Of Variance (ANOVA) test using SAS 9.2. Then the pair wise comparison of the treatment groups will be done by using the post hoc methods i.e. Bonferroni's or Fisher's LSD.

RESULTS

Demographic and other baseline characteristics

The mean age of the enrolled subjects was 28±4.5. The other baseline character details are summarized in table.

Table. Different demographic and baseline characteristics

Demographic data of the subjects at baseline(n=60)	
Gender(n)	Males(60)
Mean age(years)	28±4.5
Mean Weight(Kg)	70.72±10.32
Mean Height(cm)	169.9± 5.85
Mean Systolic B.P(mmHg)	120.63±7.92
Mean Diastolic B.P(mmHg)	78.57±7.16
Mean Pulse(/min)	74.42±9.4
Mean Respiratory Rate(/min)	20.32±8.48
Mean Temperature(F)	97.35±8.14
Grade Of baldness (n)	Grade II-17
	Grade IIa-12
	Grade IIIa-2
	Grade III-13
	Grade IIIv-5
	Grade IV- 2
	Grade V-4
	Grade Va-4

Efficacy Analysis

The Efficacy Analysis is discussed below separately as per the Drug Group

DRUG A -FD/SBF/MX-40

HAIR DENSITY: All the patients were advised to apply sufficient quantity of the study drug. All the patients' were advised to follow up every month for a period of 5 months. The data has been collected and the results were significantly shown for Hair Density, Hair Growth and Anagen/Telogen Ratio. The results collected have shown that the Drug A - FD/SBF/MX-40 has significant improvement in hair density. The mean hair density at baseline was 8.41±2.41 which increased significantly (p<0.05) to 12.94 at month 5 after treatment with Drug A= FD/SBF/MX-40. The mean values were 11.22±2.31 and 11.23±2.04 at month 2 and month 4 respectively. The percentage improvement of hair density from baseline to month 2, month 4 and month 5 was 33, 33.2 and 53.8 respectively.

HAIR DIAMETER: With Drug A -FD/SBF/MX-40 treatment, there was increase in the hair diameter when compared from baseline to month 2 and month 4. The mean values increased from 60.92±12.27 at baseline to 63.38±17.05, 66.6±10.9 and 66.08 at month 2, month 4 and month 5 respectively. The results at baseline and month 5 have not shown significant differences using ANOVA.

HAIR GROWTH

Hair Growth per day in mm data has been recorded for Drug A-FD/SBF/MX-40 and it has shown significant increase in hair growth at month 5 when compared from baseline. For Drug A-FD/SBF/MX-40, there is significant increase in the hair growth per day when compared from baseline to month 5. The mean value increased significantly (p< 0.05) from 0.12±0.04 at baseline to 0.17±0.05, 0.2, and 0.2 at month 2, 4 and month 5 respectively. The results at baseline and month 5 are significant as seen using ANOVA test and month 4 and month 5 were found significantly different when compared from baseline with post hoc Bonferroni's multiple comparison test. The percentage improvement of hair growth increased from baseline to month 2, month 4 and month 5 as 41, 66 and 66 respectively.

ANAGEN/TELOGEN RATIO

Anagen/Telogen ratio data recorded for Drug A-FD/SBF/MX-40 has shown significant increase in effect at Baseline to month 5. For Drug A-FD/SBF/MX-40, there is significant increase in the Anagen/telogen ratio when compared from baseline to month 5. The mean value increased significantly (p< 0.05) from 6.68±1.23 at baseline to 8±1.8, 9±1.4, and 10 at month 2, 4 and month 5 respectively. The results are significant as seen using ANOVA test and month 4 is found significantly different when compared from baseline with post hoc Bonferroni's multiple comparison tests. The percentage improvement of anagen/telogen ratio increased from baseline to 19.7, 34 and 50 at month 2, month 4 and month 5 respectively.

INVESTIGATOR GLOBAL AASESSMENT:

The results for global photography grades were assessed by the investigator using 7 point scale at month 2, Month 4 and month 5 when compared to baseline.

77.7% of the subjects have shown slight increase in the grade at Month 2 from baseline.

100% of the subjects have shown slight increase when compared from baseline at month 4 and month 5.
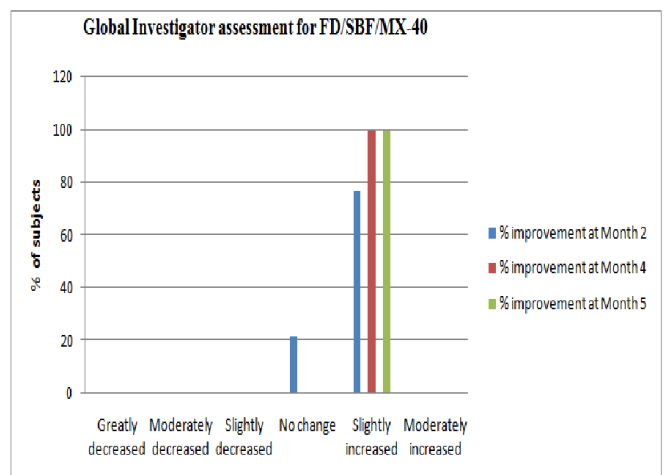

Figure. Global Investigator assessment for FD/SBF/MX-40

Table. Global photography image grading Investigator assessment

S.No	Assessment compared from baseline	% of subjects		
		Month 2	Month 4	Month 5
1	Greatly decreased	0	0	0
2	Moderately decreased	0	0	0
3	Slightly decreased	0	0	0
4	No change	22.22	0	0
5	Slightly increased	77.77	100	100
6	Moderately increased	0	0	0
7	Greatly increased	0	0	0

m-RNA estimation using RT-PCR test

There was 6.3% increase in the SHH gene mRNA expression levels after treatment over the baseline. The results were summarized in the table 9.

Table. m-RNA estimation using RT-PCR test evaluation

mRNA levels	% Improvement after month 4
FD/SBF/MX-40	6.3%

PATIENT QUESTIONNAIRE ASSESSMENT

Table: Patient questionnaire assessments for Drug A - FD/SBF/MX-40

Question	Responses	Month 1	Month 2	Month 3	Month 4	Month 5
Bald spot getting smaller	Agree	44.44%	38%	37.5%	60%	0%
	NA/DA	55.56%	50%	50%	40%	100%
	Disagree	0%	13%	12.5%	0%	0%
Appearance of hair	Better	66.67%	88%	75%	50%	0%
	No change	22.22%	13%	12.5%	50%	0%
	Best	11.11%	0%	12.5%	0%	100%
Growth of hair	Increased	44.4%	44%	75%	75%	100%
	Decreased	0%	0%	0%	0%	0%
	No change	55.5%	56%	25%	25%	0%
Effect of treatment	Effective	66.7%	75%	75%	75%	100%
	Very effective	0%	13%	0%	0%	0%
	Not effective	33.3%	13%	25%	25%	0%
Satisfaction with appearance on area	Front	55.56%	50%	62.5%	75%	0%
	Top	11.11%	25%	0%	0%	100%
	Overall	33.33%	25%	37.5%	25%	0%

Drug B- FD/SBF/PY-39

HAIR DENSITY: With Drug B- FD/SBF/PY-39 treatment, there was significant increase in the hair density when compared from baseline to month 5. The mean value was 8.65±2.19 at baseline which increased significantly (<0.05) to 14.26±3.2 at month 5. The mean values at month 2 and month 4 are 10.11±2 and 13.21±3.3 respectively. The results at baseline and month 5 are significant as seen in ANOVA and also found significantly different with post hoc Bonferroni's multiple comparison. The percentage of hair density shown from baseline to month 2, month 4 and month 5 was 16.8, 52 and 64 respectively.

HAIR DIAMETER: With Drug B- FD/SBF/PY-39 treatment, there was change in the hair diameter when compared from baseline to month 2 and month 4. The mean values changed from 66.08±15.3 at baseline to 62.36±16 and 59±8.6 at month 2 and month 4 respectively. The results at baseline and month 5 have not shown significant differences using ANOVA.

HAIR GROWTH PER DAY: For Drug B-FD/SBF/PY-39, there is significant increase in the hair growth per day when compared from baseline to month 5. The mean value increased significantly ($p < 0.05$) from 0.12±0.04 at baseline to 0.15±0.05, 0.18, and 0.2 at month 2, 4 and month 5 respectively. The results at baseline and month 5 are significant as seen using ANOVA test and month 5 is found significantly different when compared from baseline with post hoc Bonferroni's multiple comparison tests. The percentage improvement of hair growth increased from baseline to month 2, month 4 and month 5 as 25, 50 and 66 respectively.

ANAGEN/TELOGEN RATIO: For Drug B-FD/SBF/PY-39, there is significant increase in the Anagen/telogen ratio when compared from baseline to month 5. The mean value increased significantly ($p < 0.05$) from 6.58±0.90 at baseline to 7.36±0.8, 8.4±0.8 and 10.5±0.7 at month 2, 4 and month 5 respectively. The results are significant as seen using ANOVA test and month 5 is found significantly different when compared from baseline and month 2 with post hoc Bonferroni's multiple comparison tests. The percentage improvement of anagen/telogen ratio increased from baseline to month 2, month 4 and month 5 as 11, 27 and 59 respectively.

INVESTIGATOR GLOBAL ASSESSMENT

The results for global photography grades assessed by the investigator have shown increase in the appearance of the hair. At month 2, 9.09% of the subjects have shown slight decrease in the grade which decreased to 0% at month 4 and 5. At month 2, 18.18% of the subjects have shown no change in the grade which increased to 40% and 100% at month 4 and 5. At month 2, 72.72% of the subjects have shown slight increase in the grade and was 60% and 0% at month 4 and 5 respectively. The results has been given in the table 18 and figure 17

Table. Global photography image grading Investigator assessment

S.No	Assessment of subjects from baseline	% of subjects		
		Month 2	Month 4	Month 5
1	Greatly decreased	0	0	0
2	Moderately decreased	0	0	0
3	Slightly decreased	9.09	0	0
4	No change	18.18	40	100
5	Slightly increased	72.72	60	0
6	Moderately increased	0	0	0
7	Greatly increased	0	0	0
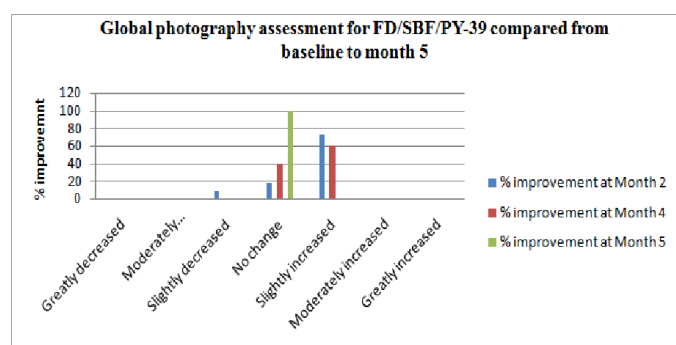
Figure. Global photography image grading Investigator

m-RNA estimation

There was 21.7% increase in the SHH gene levels in FD/SBF/PY-39 (Drug B) group after treatment.

Table. m-RNA estimation

mRNA levels	% Improvement after month 4
B- FD/SBF/PY-39	21.7%

PATIENT QUESTIONNAIRE ASSESSMENT

Table. Patient questionnaire assessments for Drug B-FD/SBF/PY-39

Question	Responses	Month 1	Month 2	Month 3	Month 4	Month 5
Bald spot getting smaller	Agree	36.36%	56%	44%	25%	50%
	NA/DA	45.45%%	33%	33%	75%	50%
	Disagree	18.18%	11%	22%	0%	0%
Appearance of hair	Better	63.64%	78%	56%	60%	50%
	No change	36.36%	11%	33%	20%	50%
	Best	0%	11%	11%	20%	0%
Growth of hair	Increased	45.45%	44%	22%	75%	50%
	Decreased	0%	0%	33%	0%	0%
	No change	54.55%	56%	44%	25%	50%
Effect of treatment	Effective	63.64%	78%	67%	75%	100%
	Very effective	0%	0%	0%	0%	0%
	Not effective	36.36%	22%	33%	25%	0%
Satisfaction with appearance on area	Front	54.55%	44%	11%	75%	50%
	Top	27.27%	44%	33%	0%	0%
	Overall	18.18%	11%	56%	25%	50%

DRUG C- FD/WBF/MX-36

HAIR DENSITY: With Drug C- FD/WBF/MX-36 treatment, there was significant increase in the hair density when compared from baseline to month 5. The mean value was 7.96 ± 2.27 at baseline which increased significantly (<0.05) to 12.49 at month 5. The mean values at month 2 and month 4 are 11.12 ± 1.5 and 13.92 ± 2.5 respectively. The results at baseline and month 5 are significant as seen using ANOVA test and month 2, month 4 and 5 were found significantly different from baseline with post hoc Bonferroni's multiple comparison test. The percentage of hair density shown from baseline to month 2, month 4 and month 5 was 40, 74 and 56 respectively.

HAIR DIAMETER: With Drug C- FD/WBF/MX-36 treatment, there was increase in the hair diameter when compared from baseline to month 2 and month 4. The mean values increased from 63 ± 13.2 at baseline to 71.63 ± 11.5 and $58.06\pm12.$at month 2 and month 4 respectively. The results at baseline and month 5 have shown significant differences (p <0.05) using ANOVA.

HAIR GROWTH PER DAY: For Drug C-FD/WBF/MX-36, there was significant increase in the hair growth per day when compared from baseline to month 5. The mean value increased significantly (p< 0.05) from 0.12 ± 0.04 at baseline to 0.13 ± 0.05, 0.19, and 0.2 at month 2, 4 and month 5 respectively. The results at month 4 and month 5 are significant when compared from baseline, month 2 and 5 results, month 1 and 5 results are found significantly different with post hoc Bonferroni's multiple comparison tests. The percentage improvement of hair growth increased from baseline to 8.3, 58.3 and 66 at month 2, month 4 and month 5 respectively.

ANAGEN/TELOGEN RATIO: For Drug C-FD/WBF/MX-36, there is significant increase in Anagen/telogen ratio when compared from baseline to month 5. The mean value increased significantly (p< 0.05) from 6.67 ± 0.78 at baseline to 7.13 ± 0.3, 8.86 ± 1.2, and 10.25 ± 0.5 at month 2, 4 and month 5 respectively. The results are significant when compared from baseline to month 5. With post hoc Bonferroni's multiple comparison test month 2, 4 and 5 results have shown significant increase. The percentage improvement of anagen/telogen ratio increased from baseline to month 2, month 4 and month 5 as 6.8, 32 and 53 respectively.

INVESTIGATOR GLOBAL ASSESSMENT

The results for global photography grades assessed by the investigator have shown increase in the appearance of the hair. At month 2, 12.5% of the subjects have shown slight decrease in the grade which decreased to 0% at month 4 and 5. At month 2, 12.5% of the subjects have shown no change in the grade which decreased to 0% and 0% at month 4 and 5. At month 2, 75% of the subjects have shown slight increase in the grade and increased to 100% and 100% at month 4 and 5 respectively.

Table. Global photography image grading Investigator assessment

S.No	Assessment from baseline	% of subjects		
		Month 2	Month 4	Month 5
1	Greatly decreased	0	0	0
2	Moderately decreased	0	0	0
3	Slightly decreased	12.5	0	0
4	No change	12.5	0	0
5	Slightly increased	75	100	100
6	Moderately increased	0	0	0
7	Greatly increased	0	0	0
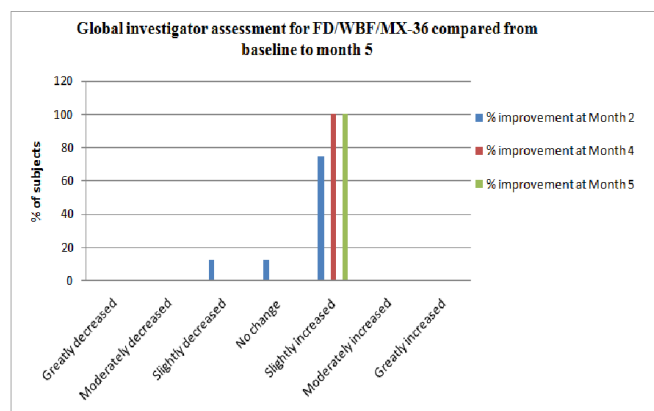
Figure. Global Investigator assessment for FD/WBF/MX-36

m-RNA estimation

There was 18% increase in the SHH gene levels in FD/WBF/MX-36 (Drug C) group after treatment.

Table. m-RNA estimation

mRNA levels	% Improvement after month 4
C- FD/WBF/MX-36	18%

PATIENT QUESTIONNAIRE ASSESSMENT

Table. Patient questionnaire assessments for Drug C-FD/WBF/MX-36

Question	Responses	Month 1	Month 2	Month 3	Month 4	Month 5
Bald spot getting smaller	Agree	30%	0%	33.3%	42.9%	25%
	NA/DA	40%	88%%	66.7%	42.9%	75%
	Disagree	30%	12%	0%	14.2%	0%
Appearance of hair	Better	60%	38%	44.4%	71.4%	100%
	No change	20%	38%	44.4%	28.6%	0%
	Best	20%	25%	11.2%	0%	0%
Growth of hair	Increased	40%	50%	44.4%	57.1%	100%
	Decreased	0%	0%	11.1%	14.3%	0%
	No change	60%	50%	44.4%	28.6%	0%
Effect of treatment	Effective	60%	63%	44.4%	28.6%	100%
	Very effective	0%	0%	0%	14.3%	0%
	Not effective	40%	37%	55.6%	57.1%	0%
Satisfaction with appearance on area	Front	40%	63%	66%	71.4%	100%
	Top	20%	52%	0%	14.3%	0%
	Overall	40%	13%	34%	14.3%	0%

DRUG D- FD/WBF/PY-37

HAIR DENSITY: With drug D-FD/WBF/PY-37 treatment, there was significant increase in the hair density when compared from baseline to month 5. The mean value was 7.96±2.27 at baseline which significantly increased (<0.05) to 12.49 at month 5. The mean values at month 2 and month 4 are 8.31±2.4 and 13.8±1.1 respectively. The results at baseline and month 5 have shown significant difference using ANOVA test. The percentage of hair density shown from baseline to month 2, month 4 and month 5 was 20, 21 and 66 respectively.

HAIR DIAMETER: With Drug D- FD/WBF/PY-37 treatment, there was increase in the hair diameter when compared from baseline to month 2 and month 4. The mean values increased from 61.75±13.5 at baseline to 64.64±4.2 and 63± 0 at month 2 and month 5 respectively. The results at baseline and month 5 have not shown significant differences using ANOVA.

HAIR GROWTH RATE: For Drug D-FD/WBF/PY-37, there is significant increase in the hair growth per day when compared from baseline to month 5. The mean value increased significantly (p< 0.05) from 0.1 at baseline to 0.15±0.05, 0.2, and 0.2 at month 2, 4 and month 5 respectively. The results at all months are significant when compared from baseline using ANOVA test and significantly different with post hoc Bonferroni's multiple comparison test. The percentage improvement of hair growth increased from baseline to month 2, month 4 and month 5 as 50, 100 and 100 respectively.

ANAGEN/TELOGEN RATIO

For Drug D-FD/WBF/PY-37, there is significant increase in Anagen/telogen ratio when compared from baseline to month 5. The mean value increased significantly (p< 0.05) from 6.73±0.90 at baseline to 7.82±1.2, 8.86±1.2, and 10 at month 2, 4 and month 5 respectively. The results at all months are significant when compared from baseline using ANOVA test and significantly different with post hoc Bonferroni's multiple comparison test for baseline and month 4. The percentage improvement of anagen/telogen ratio increased from baseline to month 2, month 4 and month 5 as 16, 31 and 48 respectively.

INVESTIGATOR GLOBAL ASSESSMENT

Table. Global photography image grading Investigator assessment at Month 2, Month 4 and Month 5 to assess hair regrowth

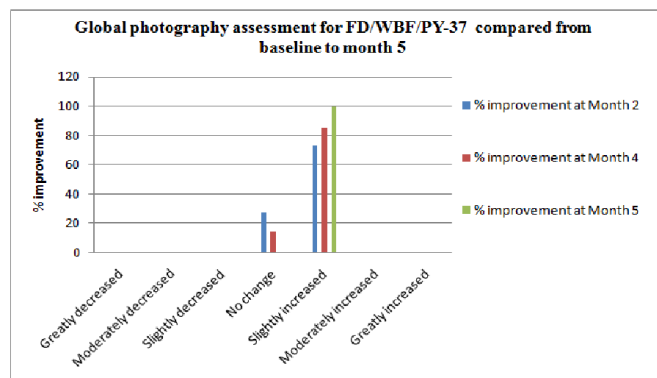| S.No	Assessment	% of subjects		
		Month 2	Month 4	Month 5
1	Greatly decreased	0	0	0
2	Moderately decreased	0	0	0
3	Slightly decreased	0	0	0
4	No change	27.27	14.28	0
5	Slightly increased	72.73	85.71	100
6	Moderately increased	0	0	0
7	Greatly increased	0	0	0

Figure. Global photography image grading Investigator assessment at Month 2, Month 4 and Month 5 to assess hair regrowth for FD/WBF/PY-37

m-RNA estimation

There was 13% increase in the SHH gene levels in FD/WBF/PY-37 (Drug D) group after treatment.

Table. m-RNA estimation

mRNA levels	% Improvement after month 4
FD/WBF/PY-37	13.5%

PATIENT QUESTIONNAIRE ASSESSMENT

Table. Patient questionnaire assessments for D -FD/WBF/PY-37

Question	Responses	Month 1	Month 2	Month 3	Month 4	Month 5
Bald spot getting smaller	Agree	50%	0%	70%	71.4%	40%
	NA/DA	30%	10%	10%	14.3%	40%
	Disagree	20%	30%	20%	14.3%	20%
Appearance of hair	Better	60%	60%	80%	57.1%	50%
	No change	30%	30%	10%	28.6%	0%
	Best	10%	10%	10%	14.3%	50%
Growth of hair	Increased	30%	50	80%	71.4%	50%
	Decreased	0%	0%	0%	0%	0%
	No change	70%	50%	20%	28.6	50%
Effect of treatment	Effective	100%	80%	90%	71.4%	100%
	Very effective	0%	10%	0%	14.3%	0%
	Not effective	0%	10%	10%	14.3%	0%
Satisfaction with appearance on area	Front	50%	50%	30%	14.3%	0%
	Top	30%	40%	30%	14.3%	0%
	Overall	20%	10%	40%	71.4%	100%

DRUG E- FD/WBF/KX-38

HAIR DENSITY: There was significant increase in the hair density when compared from baseline to month 5. The mean value was 7.36±2.43 at baseline which increased significantly (<0.05) to 12.45 at month 5. The mean values at month 2 and

month 4 are 11±3.71 and 12.45±3.3 respectively. The results at baseline and month 5 are significant as seen using ANOVA test. The percentage of hair density shown from baseline to month 2, month 4 and month 5 was 49, 75 and 65 respectively.

HAIR DIAMETER: There was increase in the hair diameter when compared from baseline to month 2 and month 4. The mean values increased from 66.25±11.6at baseline to 68.13±7.3 and 72±2.83 at month 2 and month 5 respectively. The results at baseline and month 5 have not shown significant differences using ANOVA.

HAIR GROWTH RATE: There is significant increase in the hair growth per day when compared from baseline to month 5. The mean value increased significantly ($p < 0.05$) from 0.1 at baseline to 0.16±0.05, 0.2, and 0.2 at month 2, 4 and month 5 respectively. The results at all months are significant when compared from baseline using ANOVA test and significantly different with post hoc Bonferroni's multiple comparison test. The percentage improvement of hair growth increased from baseline to month 2, month 4 and month 5 as 60, 100 and 100 respectively.

ANAGEN/TELOGEN RATIO: There is significant increase in Anagen/telogen ratio when compared from baseline to month 5. The mean value increased significantly ($p < 0.05$) from 6.51±0.78 at baseline to 7.36±1.3, 8±0.7, and 9.5±0.7 at month 2, 4 and month 5 respectively. The results at all months are significant when compared from baseline using ANOVA test and significantly increased from baseline to month 5 with post hoc Bonferroni's multiple comparison test. The percentage improvement of anagen/telogen ratio increased from baseline to month 2, month 4 and month 5 as 13, 22 and 45.9 respectively.

INVESTIGATOR GLOBAL ASSESSMNENT

The results for global photography grades assessed by the investigator have shown increase in the appearance of the hair. At month 2, 12.5%% of the subjects have shown slight decrease in the grade which decreased to 0% and 0% at month 4 and 5. At month 2, 12.5% of the subjects have shown no change in the grade and it decreased to 0% and 0% at month 4 and 5 respectively. At month 2, 75% of the subjects have shown slight increase in the grade and it increased to 100% and10 0% at month 4 and 5 respectively.

Table. Global photography image grading Investigator assessment at Month 2, Month 4 and Month 5 to assess hair regrowth

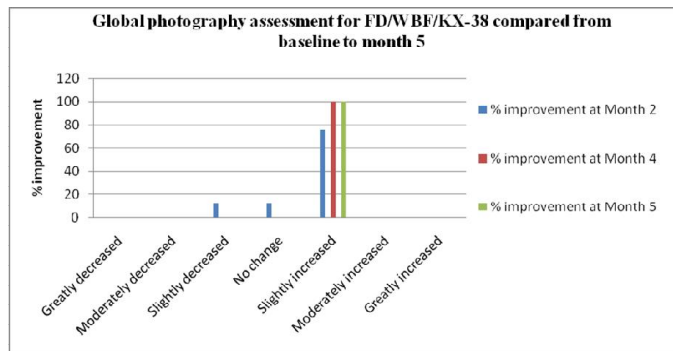| S.No	Assessment	% improvement at Month 2	% improvement at Month 4	% improvement at Month 5
1	Greatly decreased	NA	NA	NA
2	Moderately decreased	NA	NA	NA
3	Slightly decreased	12.5%	NA	NA
4	No change	12.5	0%	0%
5	Slightly increased	75%	100%	100%
6	Moderately increased	NA	NA	NA
7	Greatly increased	NA	NA	NA

Figure. Global photography image grading Investigator assessment

m-RNA estimation

There was 12.6% increase in the SHH gene levels in FD/WBF/KX-38 (Drug E) group after treatment.

Table: m-RNA estimation

mRNA levels	% Improvement after month 4
FD/WBF/KX-38	12.6%

PATIENT QUESTIONNAIRE ASSESSMENT

Table. Patient questionnaire assessments for drug E - FD/WBF/KX-38

Question	Responses	Month 1	Month 2	Month 3	Month 4	Month 5
Bald spot getting smaller	Agree	0%	22%	44.4%	20%	50%
	NA/DA	87.5%	67%	55.6%	40%%	50%
	Disagree	12.5%	11%	0%	40%	0%
Appearance of hair	Better	25%	56%	55.6%	40%	50%
	No change	37.5%	33%	44.4%	40%	50%
	Best	37.5%	11%	0%	20%	0%
Growth of hair	Increased	50%	89%	60%	75%	50%
	Decreased	0%	0%	20%	0%	0%
	No change	50%	11%	40%	25%	50%
Effect of treatment	Effective	62.5%	89%	88.9%	75%	100%
	Very effective	0%	0%	0%	0%	0%
	Not effective	37.5%	11%	11.1%	25%	0%
Satisfaction with appearance on area	Front	75%	45%	55%	40%	50%
	Top	25%	33%	33%	40%	0%
	Overall	0%	22%	12%	20%	50%

OVERALL CONCLUSION

For hair density all the five drugs have shown significant improvement when compared from baseline to month 5. The drug FD/SBF/MX-40 (Drug A) has shown significant increase in the mean hair density which increased from 8.4 at baseline to 12.9 at month 5. The percentage of hair density improvement from baseline to month 2, month 4 and month 5 was 33, 33.2 and 53.8 respectively. The drug FD/SBF/PY-39 (Drug B) has shown significant increase in the mean hair density which increased from 8.6 at baseline to 14.2 at month 5. The percentage of hair density shown from baseline to month 2, month 4 and month 5 was 16.8, 52 and 64 respectively. The drug FD/WBF/MX-36 (Drug C) has shown significant increase in the mean hair density which increased from 7.9 at baseline to 12.4 at month 5. The percentage of hair density improvement from baseline to month 2, month 4 and

month 5 was 40, 74 and 56 respectively. The drug FD/WBF/PY-37 (Drug D) has shown significant increase in the mean hair density which increased from 8 at baseline to 13 at month 5. The percentage of hair density improvement from baseline to month 2, month 4 and month 5 was 20, 21 and 66 respectively. The drug FD/WBF/KX-38 (Drug E) has shown significant increase in the mean hair density which increased from 7 at baseline to 12 at month 5. The percentage of hair density improvement from baseline to month 2, month 4 and month 5 was 49, 75 and 65 respectively. For hair diameter all the drugs FD/SBF/MX-40 (Drug A), FD/WBF/PY-37 (Drug D) and FD/WBF/KX-38 (Drug E) have shown improvement when compared from baseline. For hair growth all the five drugs have shown significant improvement when compared from baseline to month 5. The drug FD/SBF/MX-40 (Drug A) has shown significant increase in the mean hair growth rate which increased from 0.12 at baseline to 0.2 at month 5. The drug FD/SBF/PY-39 (Drug B) has shown significant increase in the mean hair growth rate which increased from 0.12 at baseline to 0.2 at month 5. The percentage improvement of hair growth increased from baseline to month 2, month 4 and month 5 as 25, 50 and 66 respectively. The drug FD/WBF/MX-36 (Drug C) has shown significant increase in the mean hair growth rate which increased from 0.12 at baseline to 0.2 at month 5. The percentage improvement of hair growth increased from baseline to month 2, month 4 and month 5 as 8.3, 58.3 and 66 respectively.

The drug FD/WBF/PY-37 (Drug D) has shown significant increase in the mean hair growth rate which increased from 0.1 at baseline to 0.2 at month 5. The percentage improvement of hair growth increased from baseline to month 2, month 4 and month 5 as 50, 100 and 100 respectively. The drug FD/WBF/KX-38 (Drug E) has shown significant increase in the mean hair growth which increased from 0.1 at baseline to 0.2 at month 5. The percentage improvement of hair growth increased from baseline to month 2, month 4 and month 5 as 60, 100 and 100 respectively. For Anagen/Telogen ratio all the five drugs have shown significant improvement when compared from baseline to month 5. The drug FD/SBF/MX-40 (Drug A) has shown significant increase in the Anagen/Telogen ratio which increased from 6.6 at baseline to 10 at month 5. The drug FD/SBF/PY-39 (Drug B) has shown significant increase in the Anagen/Telogen which increased from 6.5 at baseline to 10.5 at month 5. The drug FD/WBF/MX-36 (Drug C) has shown significant increase in the Anagen/Telogen which increased from 6.6 at baseline to 10.25 at month 5. The drug FD/WBF/PY-37 (Drug D) has shown significant increase in the mean Anagen/Telogen ratio which increased from 6.7 at baseline to 10 at month 5. The drug FD/WBF/KX-38 (Drug E) has shown significant increase in the mean Anagen/Telogen ratio which increased from 6.5 at baseline to 9.5 at month 5.

The results for global photography grades assessed by the investigator have shown increase in the appearance of the hair. At month 2, 22.2% of the subjects have shown no change in the grade which decreased to 0% at month 4 and 5. At month 2, 77.7% of the subjects have shown slight increase in the grade which increased to 100% at month 4 and 5. The results for global photography grades assessed by the investigator have shown increase in the appearance of the hair. At month 2, 9.09% of the subjects have shown slight decrease in the grade

which decreased to 0% at month 4 and 5. At month 2, 18.18% of the subjects have shown no change in the grade which increased to 40% and 100% at month 4 and 5. At month 2, 72.72% of the subjects have shown slight increase in the grade and was 60% and 0% at month 4 and 5 respectively. The results for global photography grades assessed by the investigator have shown increase in the appearance of the hair. At month 2, 9.09% of the subjects have shown slight decrease in the grade which decreased to 0% at month 4 and 5. At month 2, 18.18% of the subjects have shown no change in the grade which increased to 40% and 100% at month 4 and 5. At month 2, 72.72% of the subjects have shown slight increase in the grade and was 60% and 0% at month 4 and 5 respectively

The results for global photography grades assessed by the investigator have shown increase in the appearance of the hair. At month 2, 12.5% of the subjects have shown slight decrease in the grade which decreased to 0% at month 4 and 5. At month 2, 12.5% of the subjects have shown no change in the grade which decreased to 0% and 0% at month 4 and 5. At month 2, 75% of the subjects have shown slight increase in the grade and increased to 100% and 100% at month 4 and 5 respectively. The results for global photography grades assessed by the investigator have shown increase in the appearance of the hair. At month 2, 12.5% of the subjects have shown slight decrease in the grade which decreased to 0% at month 4 and 5. At month 2, 12.5% of the subjects have shown no change in the grade which decreased to 0% and 0% at month 4 and 5. At month 2, 75% of the subjects have shown slight increase in the grade and increased to 100% and 100% at month 4 and 5 respectively.

The subjects were given a monthly questionnaire to determine qualitatively the status of their hair loss and new hair growth, as well as the presence of side effects. There were 5 questions in the questionnaire which asked about the satisfaction of the subjects with respect to bald spot getting smaller, appearance of hair, improvement in hair growth, slowing down hair loss and satisfaction with the hair. There was improvement in m-RNA SHH gene levels in all the 5 groups when compared from baseline. There was 6.3% increase in the SHH gene levels in FD/SBF/MX-40 (Drug A) group after treatment. There was 21.7% increase in the SHH gene levels in FD/SBF/PY-39 (Drug B) group after treatment. There was 18% increase in the SHH gene levels in FD/WBF/MX-36 (Drug C) group after treatment. There was 13% increase in the SHH gene levels in FD/WBF/PY-37 (Drug D) group after treatment. There was 12.6% increase in the SHH gene levels in FD/WBF/KX-38 (Drug E) group after treatment. No serious adverse events were reported. All Laboratory parameters were found normal values at screening and post study visit for all subjects. All the drugs were tolerated well. The reported adverse events were Headache and Itching.

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