Development of effective & safe products for Hair care

6th International Conference on Cosmetology, Trichology & Aesthetic Practices

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DABUR
RESEARCH
FOUNDATION

- Established in 1884, Dabur India Ltd is among the oldest and largest healthcare company in India

- Has over 5000 employees working in more than 20 countries

- Market Cap of over 4 bn USD, DIL recently achieved sales of 1 bn USD

- More than 600 herbal products in market

- 17 ultra-modern manufacturing units spread around the globe

- Products marketed in over 60 countries

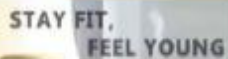- More than 5000 distributors and over 2.8 million retail outlets all over India
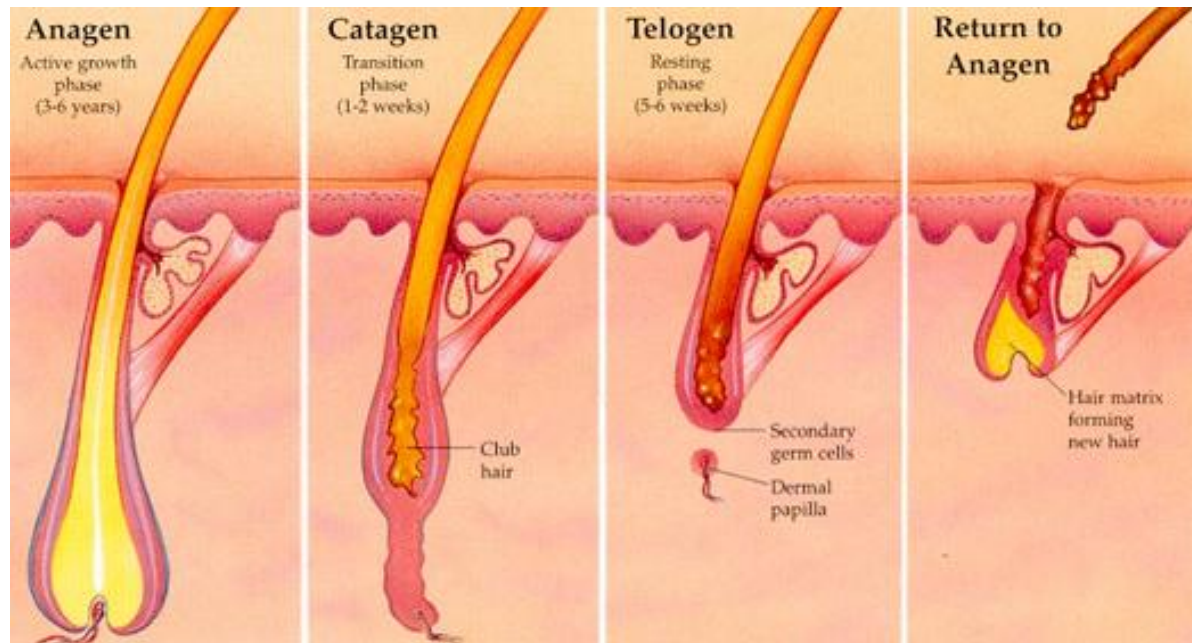
- ❑ Indian Contract Research Organization focused on Preclinical drug discovery & Development

- ❑ Led the R & D programs of Dabur group of companies (1979 -2008)

- ❑ **Strategic spin off of the parent group in year 2008 to become a Contract Research Organization in the niche area of Preclinical development**

- ❑ Positioned as a Biology specialist CRO with services in several therapeutic areas

- ❑   More than 20 years of experience in preclinical development of Phytochemicals, botanical extracts & biologically targeted products

- ❑ Comprehensive Services in Cell Biology, Pharmacology, Toxicology, DMPK, Bioanalytical, Analytical & formulation development to enable lead identification and product development

- ❑ Experience & knowledge of global regulatory requirements for submission of data packages

- ❑ GLP compliant studies

- ❑ Multi site facility in New Delhi, well connected to the International Airport

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- Cosmetics Regulation, which replaces the Cosmetics Directive as of 11 July 2013 establishes a prohibition to test finished cosmetic products and cosmetic ingredients on animals (testing ban), and a prohibition to market in the European Union finished cosmetic products and ingredients included in cosmetic products which were tested on animals for cosmetics purposes (marketing ban).
- Currently there is progressive opinion that products that may cause a change in skin function are on the borderline of cosmetic & drug.

- However the guidelines recommend the adoption of Alternative methods of screening for reduction in usage of animals for testing of cosmetics (the principle of 3Rs)

- Human dermal papilla cells (DPCs), keratinocytes (HaCaT) and fibroblasts (HFF-1) represent the key cell populations appropriate for screening hair-growth promoting activities of test compounds.

- The establishment of hair follicle culture models represents the interaction of keratinocytes, fibroblast and melanocytes in a 3D environment to analyze all the aspects of *in vivo* hair follicle behaviour.

- Dabur Research Foundation has taken several initiatives to minimize the usage of animals for testing of cosmetics/cosmaceuticals & have build a step wise strategy of *in vitro* , *ex vivo* & *in vivo* screens for identification of hair growth promoters.


CONNECTING THE DOTS FOR ANIMALS:
HISTORY OF THE EU BAN ON ANIMAL TESTING FOR COSMETICS

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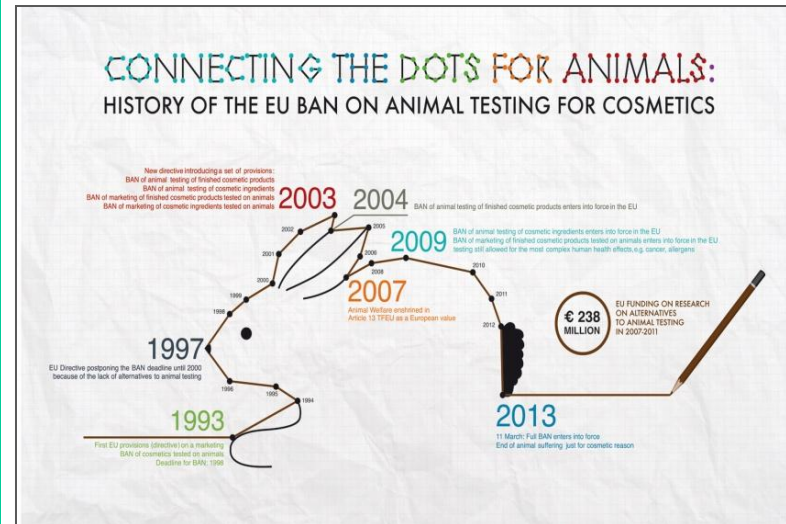
Models for identifying hair growth promoters

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A repertoire of in vitro, in vivo & ex vivo models for evaluating products for their effect on hair

Parameters :

- ❑ **Hair Growth Promotion/Androgenetic alopecia**
- ❑ **Chemotherapy induced alopecia**
- ❑ **Hair Growth inhibition**
- ❑ **Hair color pigmentation- Anti-graying/Blackening**
- ❑ **Hair fiber damage and restoration**
- ❑ **Conditioning, smoothing, softening properties**
- ❑ **Hair quality enhancers**
- ❑ **Photoprotection**
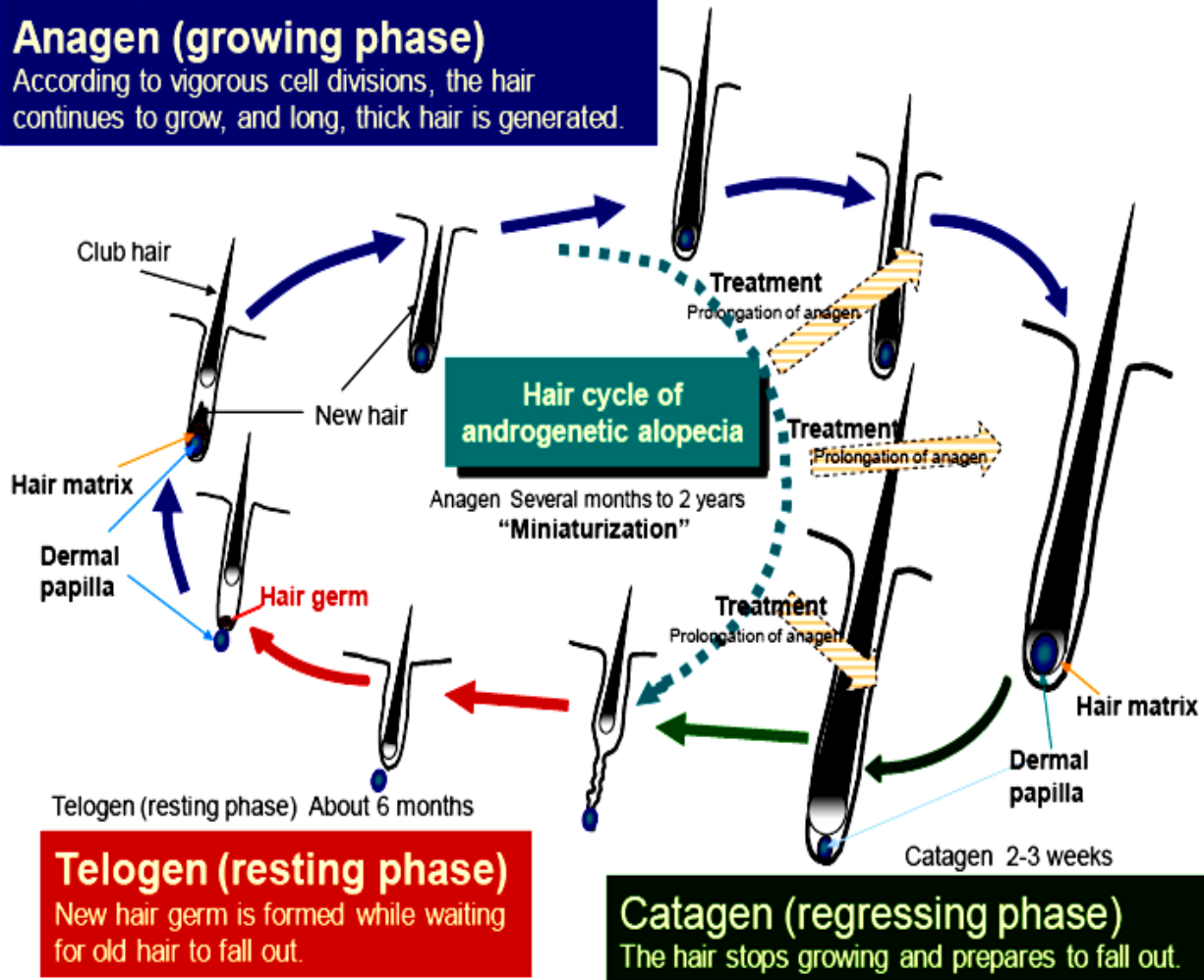- ❑ **Anti – oxidants**
- ❑ **Safety of hair products**

Healthy   Damaged
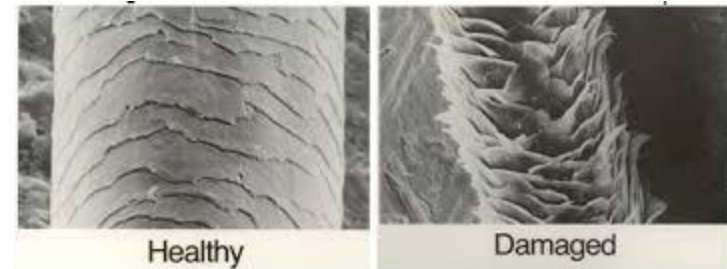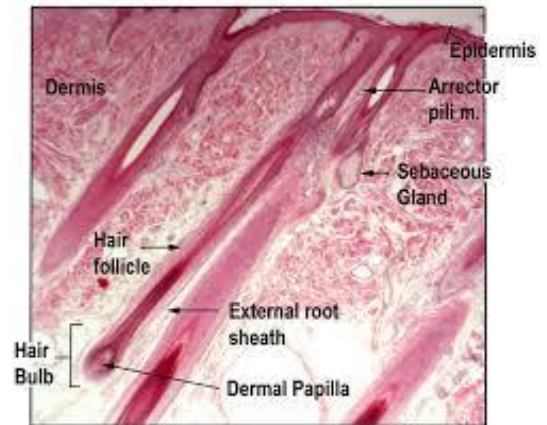
Molecular targets of novel hair growth promoters. Role of 5 α reductase and androgen receptor
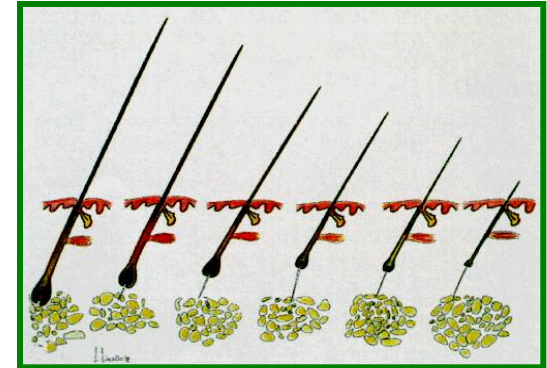
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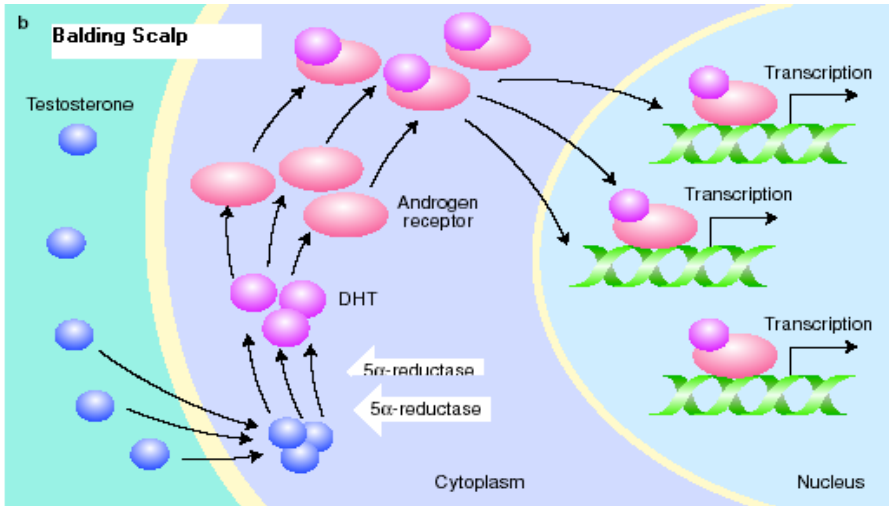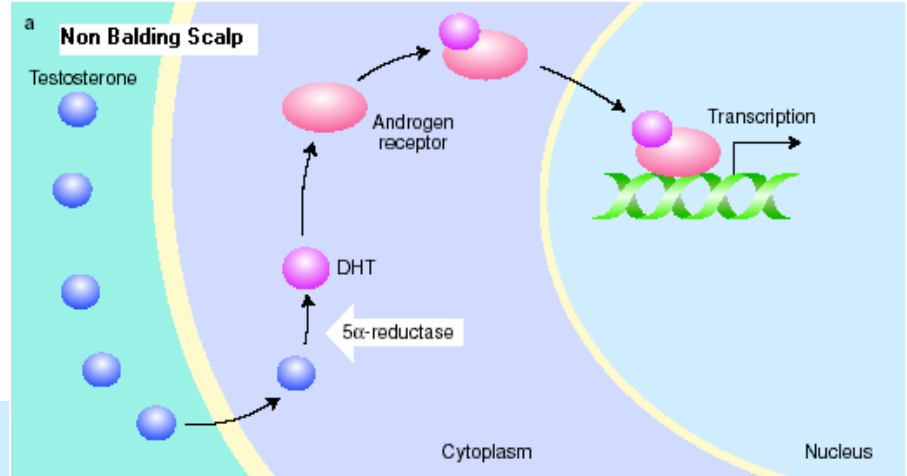
Anagen 3-7 years

Anagen (growing phase)
According to vigorous cell divisions, the hair continues to grow, and long, thick hair is generated.

Club hair

New hair

Hair matrix

Dermal papilla

Hair germ

Hair cycle of androgenetic alopecia

Anagen Several months to 2 years
"Miniaturization"

Treatment
Prolongation of anagen

Treatment
Prolongation of anagen

Treatment
Prolongation of anagen

Hair matrix

Dermal papilla

Catagen 2-3 weeks

Telogen (resting phase) About 6 months

Telogen (resting phase)
New hair germ is formed while waiting for old hair to fall out.

Catagen (regressing phase)
The hair stops growing and prepares to fall out.

(C) 2009 R-Tech Ueno, Ltd.
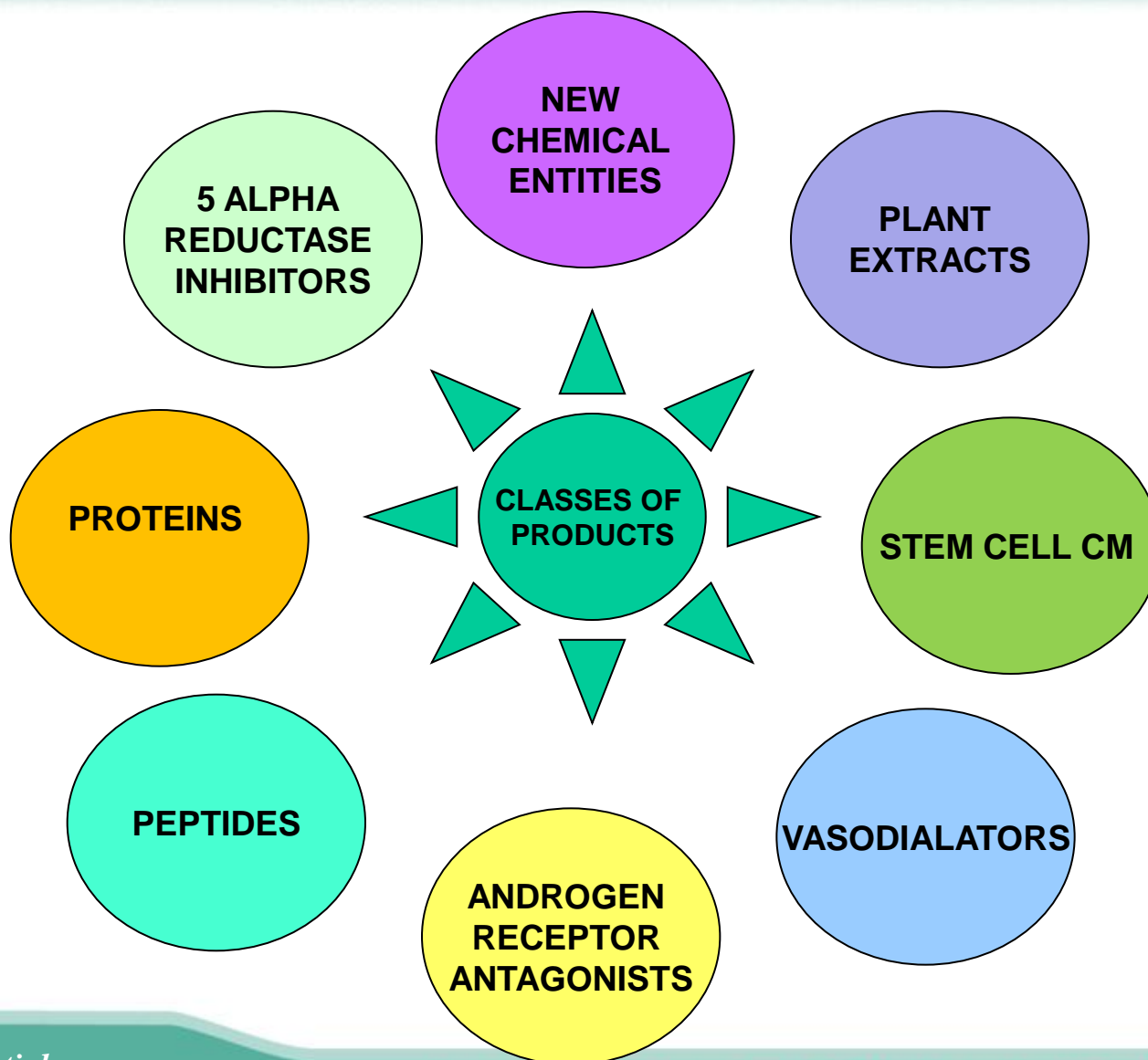
Androgenetic Alopecia

- Progressive diminution of hair shaft diameter and length in response to systemic androgens.
- Follicular miniaturization.
- Increased latency between the telogen and new anagen phase.
- Reduction in the volume of the matrix cells.

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NEW CHEMICAL ENTITIES

5 ALPHA REDUCTASE INHIBITORS

PLANT EXTRACTS

PROTEINS

CLASSES OF PRODUCTS

STEM CELL CM

PEPTIDES

ANDROGEN RECEPTOR ANTAGONISTS

VASODIALATORS

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Overall strategy for screening hair growth promoting agents

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Desirable properties of a hair growth product

S. No.	Desirable Property	Actives
1	Prolong life cycle of hair/Awakens dormant cells	Amla, Callus extracts
2	ECM stimulant for improving scalp health	Callus extracts, Coffee bean
3	Keratinocyte and fibroblast proliferator	Saw palmetto, Carrot seed oil
4	Angiogenic/Vasorelaxant	Onion juice, Grapeseed oil
5	Antioxidant	Aloevera, Coffee bean
6	Anti-inflammatory agent	Lavender oil, Thyme
7	5 alpha reductase inhibitor	Coffee bean, Saw palmetto
8	Aromatase inhibitor	Grape seed oil, Coffee bean
9	Hair nourishment	Onion juice, Thuja orientalis
10	Melanin stimulator	Capsacian, Onion juice
11	Positive effects on hair appearance	Eclipta alba, Hibiscus
12	Humectants	Jojoba oil, Alovera
13	Anti-septic/anti-dandruff	Hibiscus, Thyme
14	Removes pollutants and impurities from scalp	Jojoba oil, Carrot seed oil
15	soothing	Alovera, Thuja orientalis

Natural Hair Growth Enhancers

brigittelinford.com

ZINGIBER OFFICINALE, PANAX GINSENG, ROSEMARY, MORINGA OLEIFERA, CENTELLA ASIATICA, ALOE BARBADENSIS, LAVENDER, LEMON GRASS, PEPPERMINT, BIOTIN, VIRGIN COCONUT OIL

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3-tier strategy for screening hair growth promoters

	Relevance	**End points**	**Claim/s**
Primary screening — Keratinocytes, Fibroblasts, Dermal papilla cells	Play key roles in following: • Support and enhancement of scalp health and promotion of hair strengthening • Renewal of ECM proteins by stimulating skin remodeling and increasing size of hair follicles • Morphogenesis and growth of hair follicles	Increase in cell-proliferation • Increase in Collagen synthesis • Increase in VEGF secretion • Inhibition of IL-8 production Increase in cell-proliferation	• Hair strengthening • improved hair anchorage • Improved scalp health • Stimulates skin remodeling • Better hair anchorage • Hair growth stimulation • Hair loss prevention by inducing dermal papilla cells to reactivate hair growth
Secondary screening — Ex vivo mouse vibrissae hair follicles	• Bridges the gap between simple *in vitro* models and complex *in vivo* systems. • Play important roles in the morphogenesis and visualization of the growth of hair follicles.	Increase in hair bulb and hair shaft thickness	• Hair growth stimulation • Hair loss prevention • Hair strengthening • Hair blackening • Improved hair anchorage
Assessment using *in vivo* model — C57BL/6 or or C3H mice	Well established model for studying hair growth promotion.	• Hair growth promotion • Hair parameters - Hair pull test, hair density, hair diameter & hair bulb-thickness • Follicle count in sub cutis • Skin thickness	• Hair fall reduction • Hair growth promotion

Selected compounds (Primary → Secondary)

Selected compounds (Secondary → Assessment)

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Relevance

End points

Claim/s

Primary screening

Keratinocytes

Epidermal cells act as a counterpart population to DPCs to support and enhance scalp health and promote strengthening of hair

Increase in cell-proliferation

- Hair strengthening
- improves hair anchorage
- Scalp health improvement

Fibroblasts

Plays key role in renewal of ECM proteins by stimulating the skin remodeling, and increasing size of hair follicles

- Increase in Collagen synthesis
- Increase in VEGF secretion
- Inhibition of IL-8 production

- Stimulates skin remodeling
- Better hair anchorage

Dermal papilla cells

•Play important role in the morphogenesis and growth of hair follicles.

•The follicular proliferation and apoptosis in DPCs may be a key strategy for the control for hair growth and regression.

Increase in cell-proliferation

- Stimulation of hair growth
- Reduction of hair loss by inducing dermal papilla cells to reactivate hair growth

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Test System

Human follicle dermal papilla cells (HFDPCs)

Study Design

· **Plating of cells in complete medium for 24 h**

· **Serum starvation for 24 h**

· **Treatment of HFDPCs with test agent**

· **Incubation period : 6 days**

· **Effect on proliferation of HFDPCs by MTT assay**

Positive Controls for validation

· **Minoxidil Sulphate**

· **EGF**

· **FGF**

· **Ascorbic acid**

Claims & deliverables

· **Stimulatory effect on proliferation of HFDPCs**

· **Reduction of hair loss by inducing dermal papilla cells to reactivate hair growth**

HFDPCs
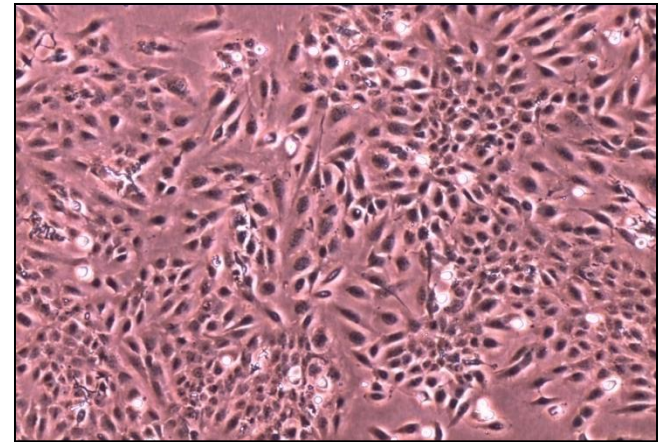
100X magnification
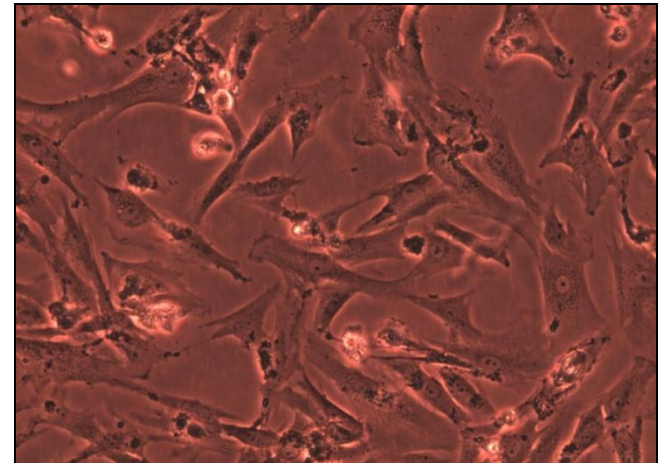
200X magnification
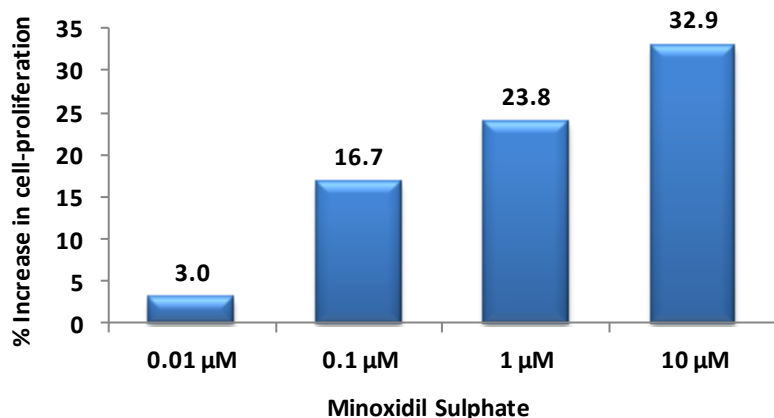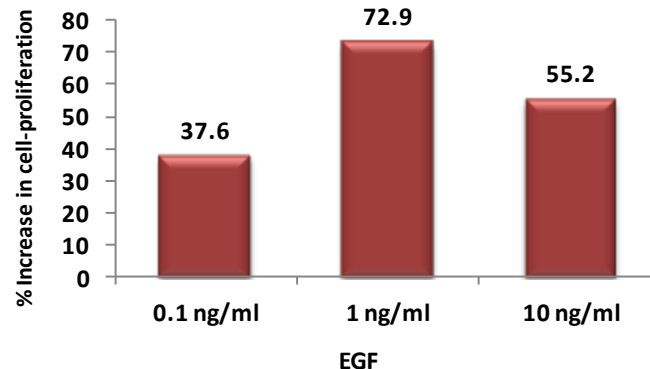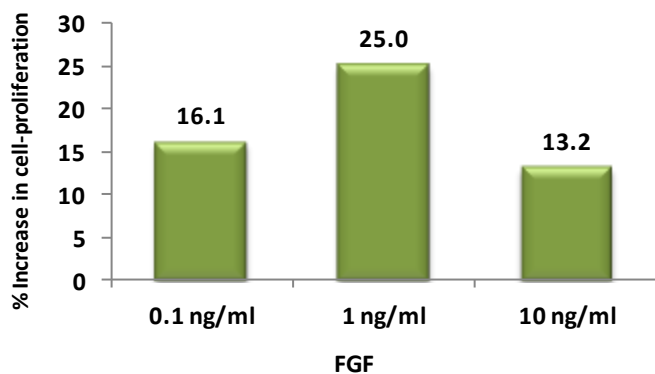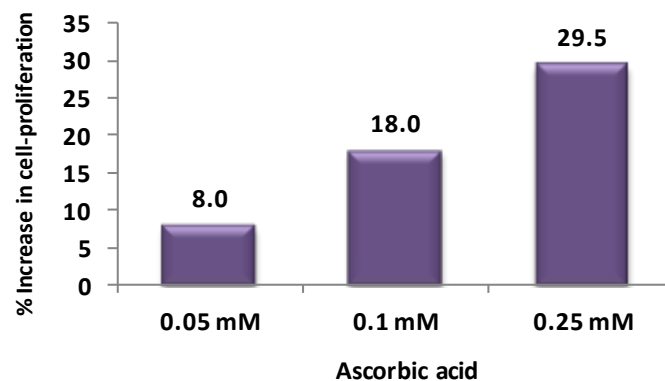
Minoxidil Sulphate, EGF, FGF and Ascorbic acid enhanced cellular proliferation of HFDPCs

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Test System

Human immortalized human keratinocyte line (HaCaT)

Study Design

- Plating of cells in complete medium for 24 h
- Serum starvation for 24 h
- Treatment of cells with test agent
- Incubation period : 6 days
- Effect on proliferation of cells by MTT assay

Positive Controls for validation

- Minoxidil Sulphate
- EGF
- FGF

Claims & deliverables

- Stimulatory effect on proliferation of HaCaT cells
- Improvement of scalp health and anchorage

properties

Morphology of HaCaT cells
100X magnification

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Minoxidil sulphate
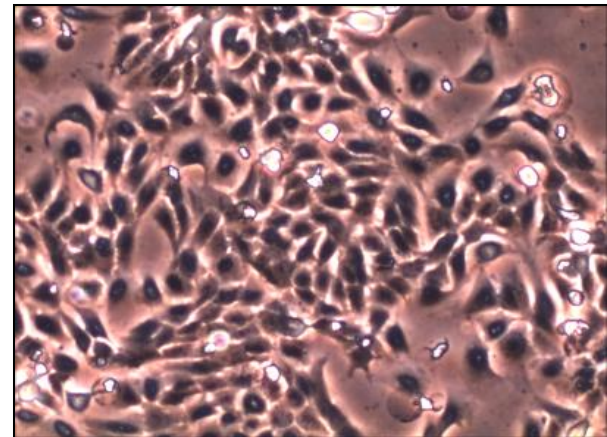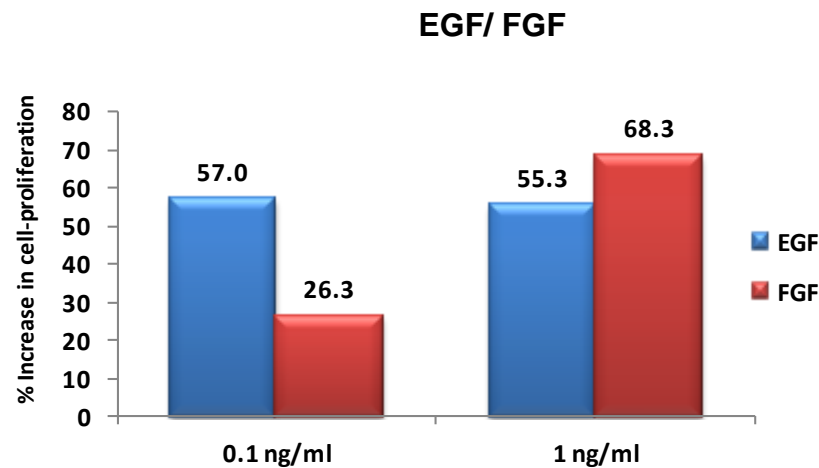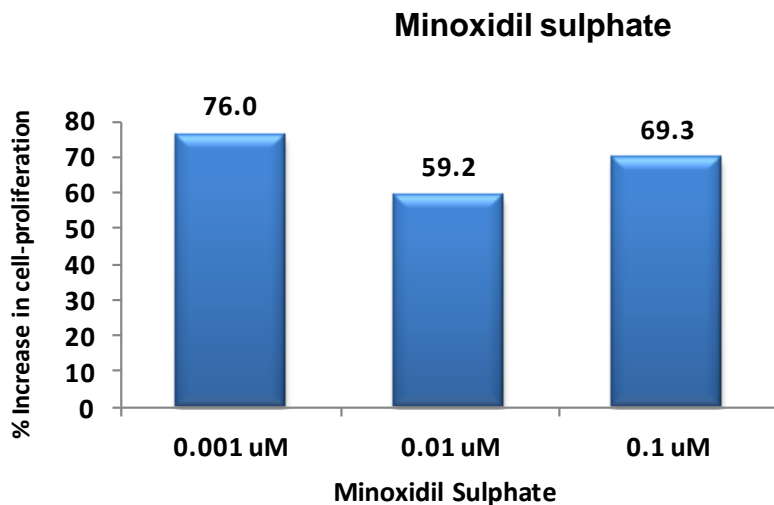
EGF/ FGF

Minoxidil Sulphate, EGF & FGF enhanced cellular proliferation of keratinocytes

• *Ex vivo* models have been used to bridge the gap between simple *in vitro* systems and complex *in vivo* models by combining the best of both the systems.

• The hair follicle organ culture model is an exceptionally accessible way to assess the interaction of epithelial (e.g. keratinocytes), mesenchymal (e.g. fibroblast) and neuroectodermal (e.g. melanocytes) cells in a 3D manner.

•This model allow us to assess hair growth modulations by various natural and pharmacological agents.

•The hair follicle culture model is representative for all the aspects of *in vivo* hair follicle behaviour.

Mouse vibrissae hair follicle

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Strategy for *ex vivo* screening

Relevance **End points** **Claim/s**

Ex-vivo screening →

- To bridge the gap between simple *in vitro and* complex *in vivo* models systems by combining the best of both systems.

- Simplifies the understanding of the specific mechanisms which can be studied with more clarity and ease.

- Play important role in the morphogenesis and visualization of the growth of hair follicles (hair bulb and hair shaft).

→ **Increase in hair bulb diameter**

- Hair strengthening
- Improved hair anchorage
- Scalp health improvement

→ **Increase in hair shaft thickness**

- Hair strengthening
- Improved hair anchorage

→ **Increase in hair shaft length**

- Stimulation of hair growth

→ **Increase in hair melanin content**

- Hair blackening

→ **Hair morphology**

- Prevention of hair loss

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Test System

Mouse vibrissae hair follicle

Study Design

- Isolation of vibrissae hair follicle *
- Maintenance in growth medium
- Treatment with test item
- Incubation period : 15 days – 20 days

Positive Control

- Caffeine
- EGCG (Epigallocatechin-3-gallate)
- Minoxidil
- Hair oil

Parameters evaluated

- Hair shaft length
- Hair shaft thickness
- Hair bulb diameter
- Melanin content

* Yield ~10 -15 follicles /animal

Dissection of whisker pad from 3 weeks old C57BL/6 mice

Isolation of follicles from the series of row of vibrissae
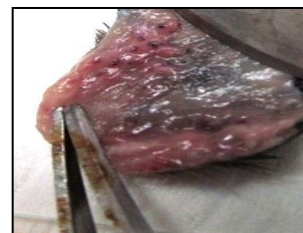
Culture of hair follicle in growth medium at 37º C and 5% CO_2.
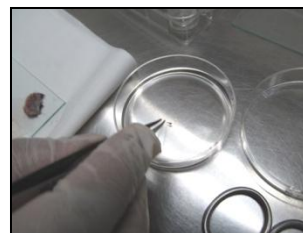
Treatment with positive control/Test compounds

Photodocumentation and analysis

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Effect of Minoxidil (0.5 mM)

Day-0	Day-11

Follicle-1

Follicle-2

Follicle-3
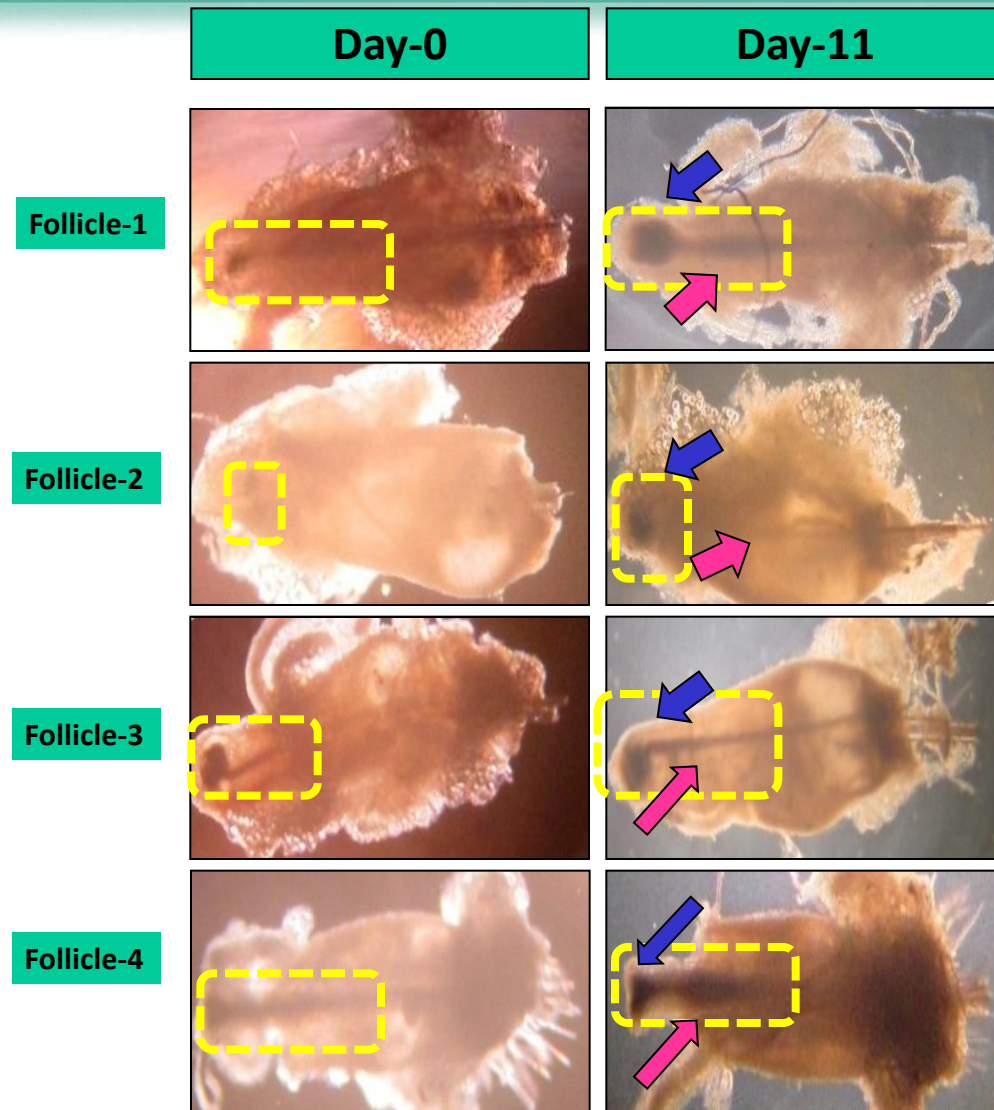
Follicle-4

➡ Increase in hair bulb diameter
➡ Increase in hair shaft thickness

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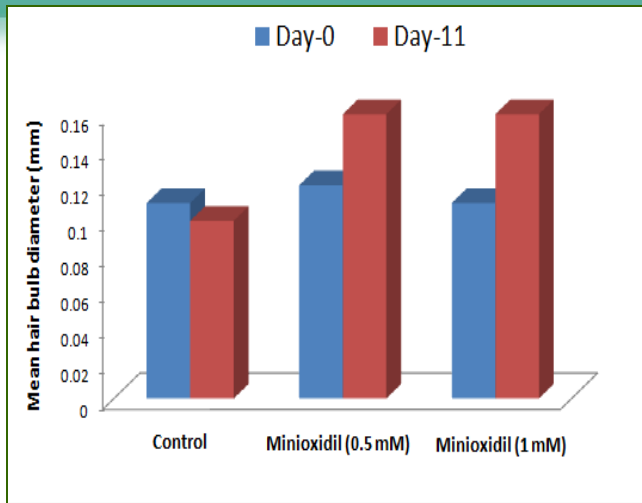
Comparative mean hair follicle bulb diameter of day 0 vs. day 11 of control and minoxidil sulphate treated groups
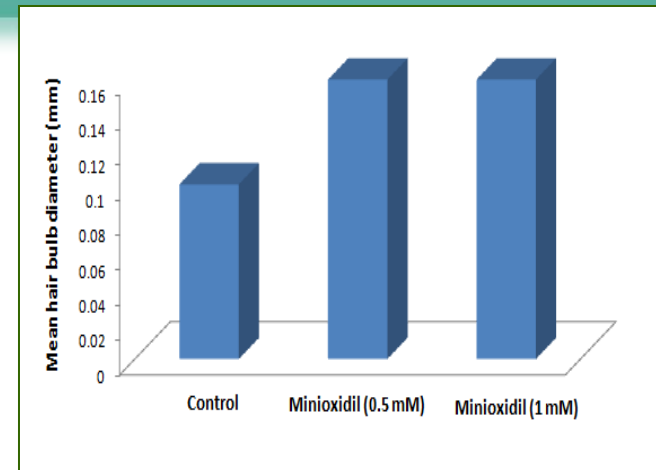
Comparative mean hair follicle bulb diameter of control and minoxidil sulphate treated groups (Day 11)
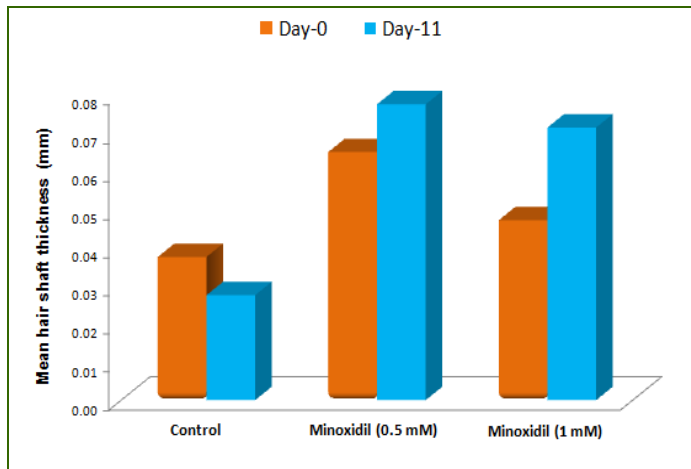
Comparative mean hair follicle shaft thickness data of day 0 vs. day 11 of control and minoxidil treated groups
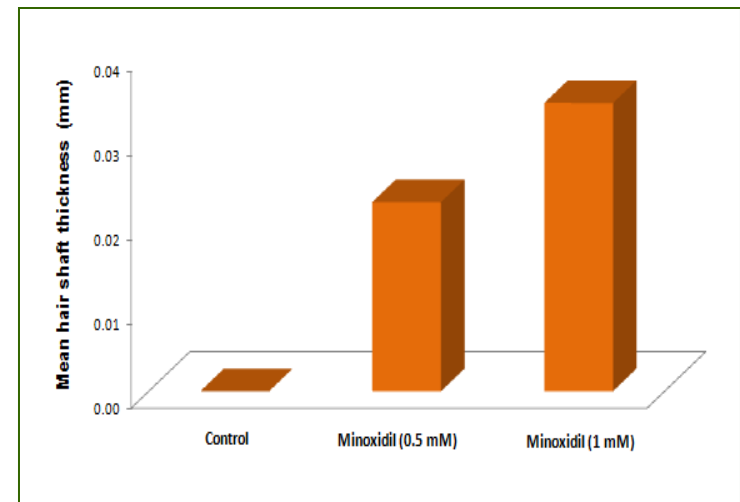
Comparative mean hair follicle shaft thickness data of control and minoxidil treated groups (Day 11)

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In vivo efficacy model for hair growth promoters

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Test System

Species	C3H/HeJ / C57BL/6 mice, age 6-9 weeks

C3H/HeJ   **C57BL/6**

Study design

➤ Selection of animals in telogen phase of hair growth

➤ Clipping of dorsal back extending to neck region

➤ Application of positive control/ test item by topical/oral/subcut route

➤ End points

1. **Visual analysis**: Percentage anagen induction, mean hair growth score, visual melanogenesis

2. **Histological analysis**: Total follicle count, follicle count in subcutis, morphometry for skin thickness

3. **Hair parameters**: Hair thickness, hair weight, hair blackening

S.No	Observation	Hair growth score
1	No hair growth, pink skin	0
2	Skin color changes from pink to gray	0.5
3	Skin color changes from gray to dark gray/black without visible hair growth, indicating the onset of anagen	1
4	Sparse hair growth	1.5
5	Diffuse short hair growth	2
6	Moderate hair growth	2.5
7	Dense, normal coat hair	3

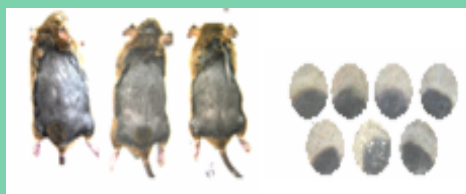**Integrated Research Solutions in Preclinical Biology**

Hair growth score
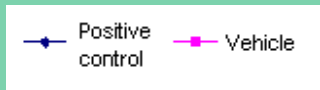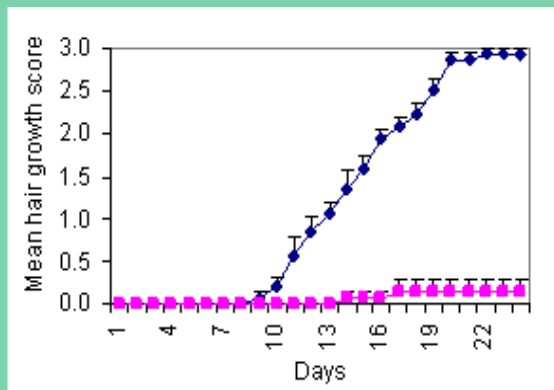
Visual melanogenesis
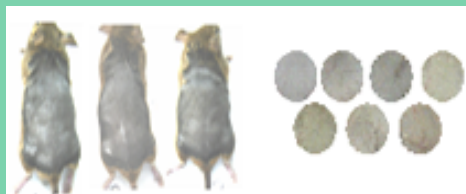
Vehicle treated group

Positive control treated group

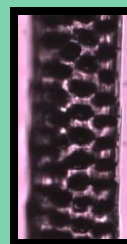## Histological analysis

Vehicle treated group

Dermis

Sub cutis
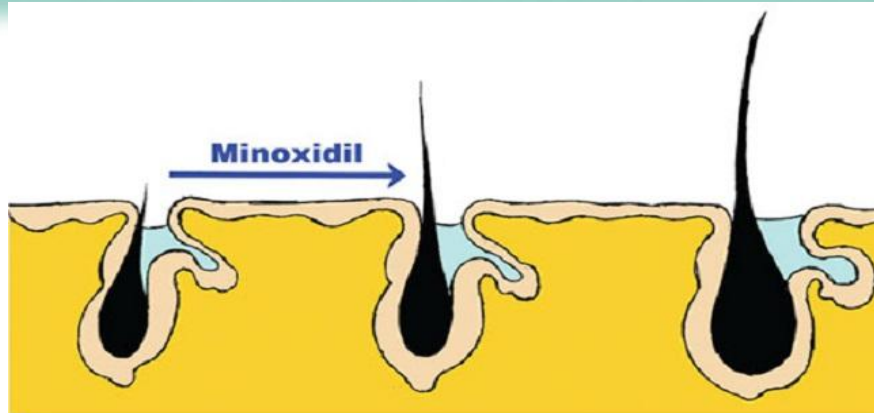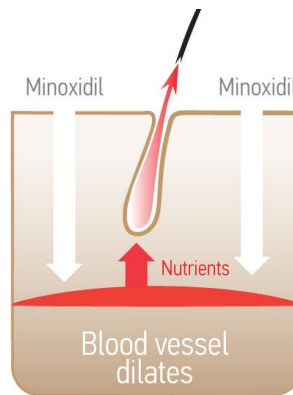
Positive control treated group

Hair thickness

Vehicle group

Positive control group

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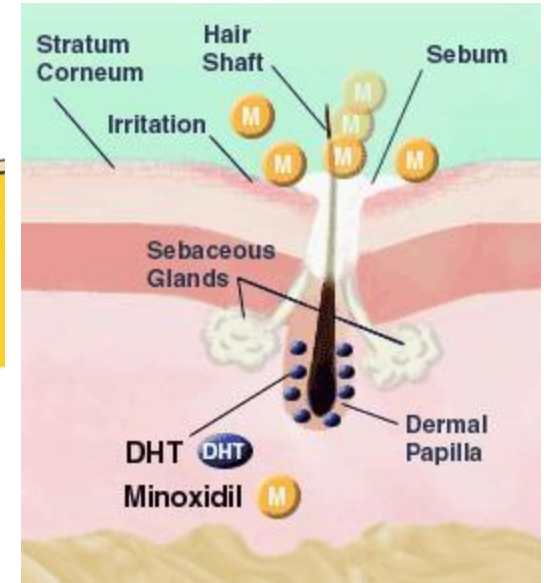
Minoxidil helps increase blood flow to hair follicle by dilating teh blood vessels. This means more nutrients get to teh hair follicle thus promoting growth.

Rogaine (Minoxidil) was the first hair growth drug approved by the FDA. Relative to placebo, a foam Minoxidil (5%) for 16 weeks is associated with 70.6% self-reported improvement against 42.4% improvement (19.2% worsening) on placebo.

Mean Hair Growth Score
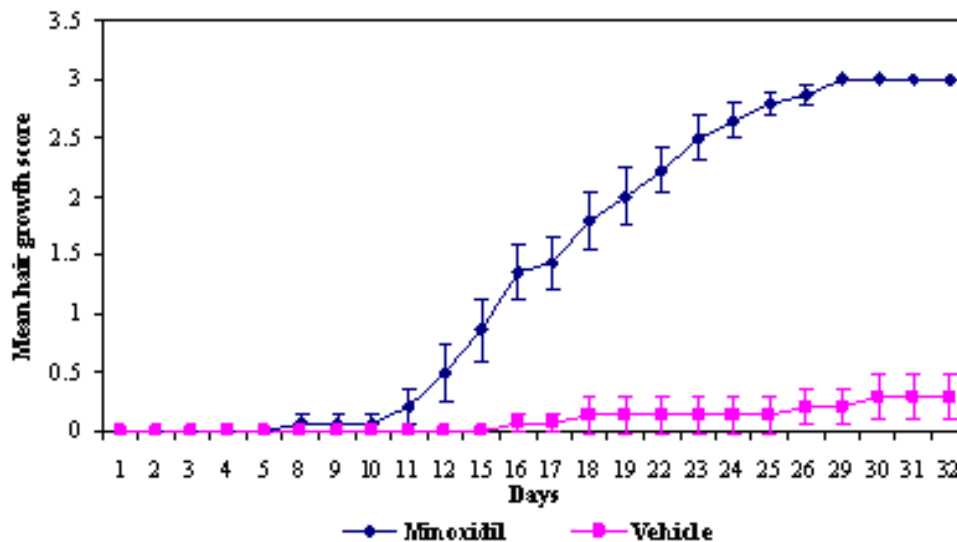
Percent anagen induction

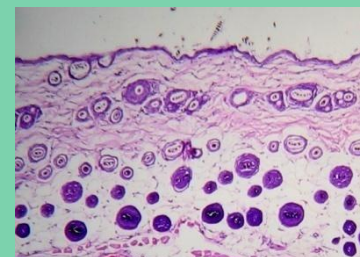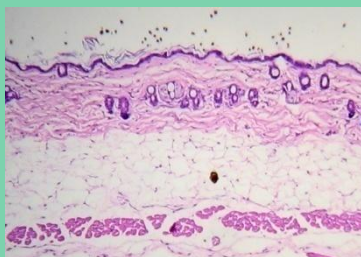| Group	Skin Color	% Anagen induction
Minoxidil	Black	100 % (7/7 animals)
Vehicle Control (Propylene Glycol:Ethanol::Water, 5:3:2)	Pink	0 % (0/7 animals)

Cont..

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Vehicle treated group

Minoxidil treated group

Visual Melanogenesis

Histological Analysis

Group	Mean ± SEM	
	Follicle Count in Subcutis (No.)	**Skin thickness**
Minoxidil	48.14 ± 3.13	370.15 ± 38.66
Vehicle	0	263.3 ± 11.68

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TEA TREE OIL FOR HAIR GROW

AMLA - MIRACLE HAIR OIL

* natural conditioner
* dandruff remedy
* hair growth
* reduce hair fall
* hair shine

THEINDIANSPOT

Dabur MAHA BHRINGRAJ HAIR OIL HUILE CAPILLAIRE

Net : 200 mL
6.76 fl.oz. U.S.
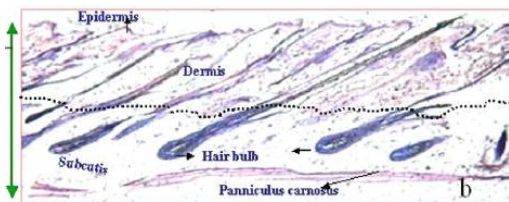
LAVENDER OIL FOR HEALTHY HAIR
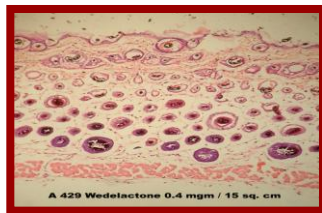
The essential oil aids in hair growth as well as treats sleeplessness, stress and anxiety. It can also help stop hair loss.
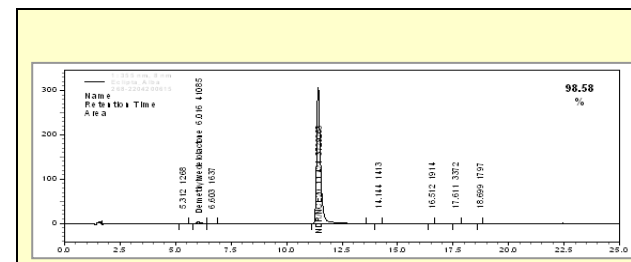
THEINDIANSPOT

PURE Follicle Growth Rx Shampoo SULFATE-FREE

PURE Follicle Growth Rx Conditioner

PURE FOLLICLE GROWTH DROPS
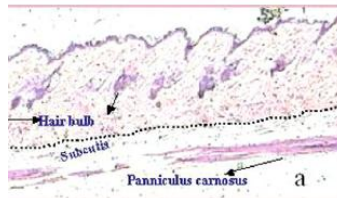
PURE FOLLICLE GROWTH TEMPLE GROW

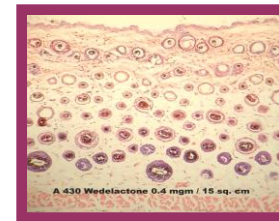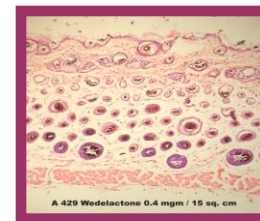**Experience with plant extracts reported to cause hair growth promotion**

Eclipta alba

Vehicle controls

DRF/NCE20

DRF/NCE20 – 95 % efficacy

Representative of studies with 10 animals/ group

J Ethnopharmacol, 124(3); 450-6 (2009)

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Andogenetic Alopecia: A Defect in Converting Stem Cells to Progenitor Cells
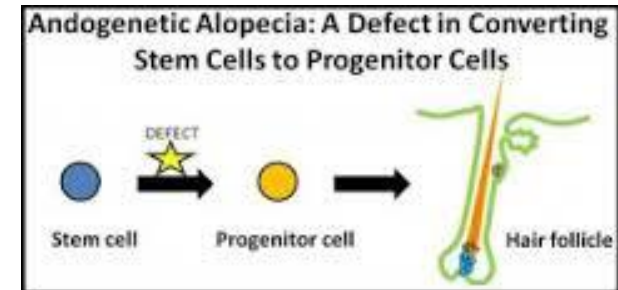
The new hair loss product containing stemoxydine molecule, a molecule that mimics the effect of hypoxia by stabilizing the protein called Hif1a. Under hypoxia culture conditions, stemoxydine will target the stem cells .i.e. " Controlling the function of stem cells and would ultimately lead to increase hair density in hair loss sufferers.
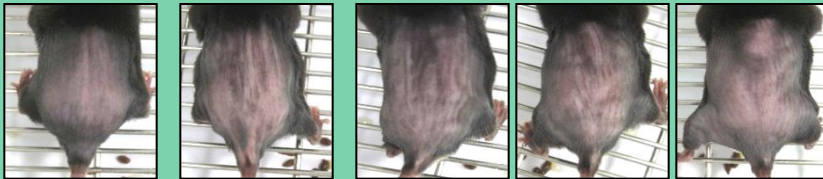
Claims:

•**Stimulate stem cell functions.**
•**Enhance Hair fiber**
•**Re-densified hair**

Clinical data:
Efficacy demonstrated on 101 subjects daily application for 3 months:
•**Increase +4% hair density**
•**1700 new hair fibers**
•**Hair appear denser (visibly)**

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Vehicle treated group

Animal 1 Hair growth score= 0
Animal 2 Hair growth score= 0
Animal 3 Hair growth score= 0
Animal 4 Hair growth score= 0
Animal 5 Hair growth score= 0

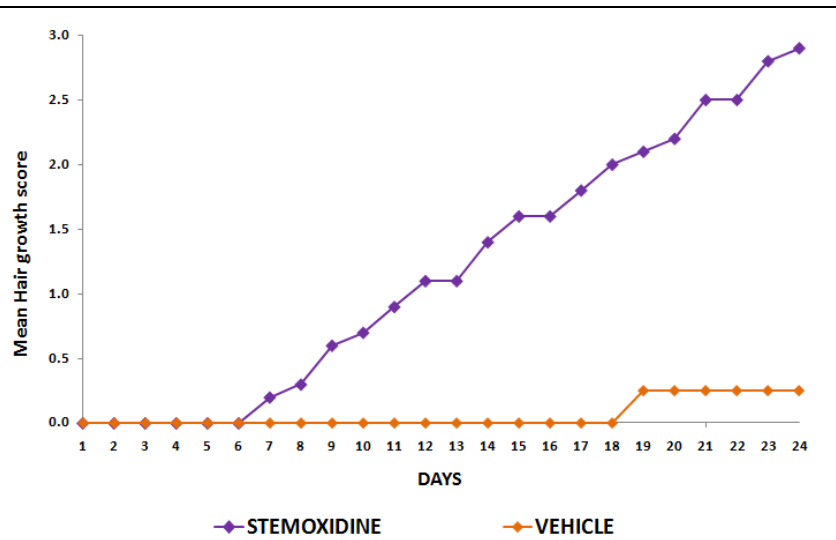**Stemoxidine treated group**

Animal 1 Hair growth score= 2
Animal 2 Hair growth score= 3
Animal 3 Hair growth score= 0.5
Animal 4 Hair growth score= 2.5
Animal 5 Hair growth score= 3

Group	Skin Color	% Anagen induction
Stemoxydine	Black	100 % (5/5 animals)
Vehicle Control	Pink	0 % (0/5 animals)

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Harnessing the power of plant stem cells to develop potent hair growth promoters

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1. Harvest of leaf tissue from explants grown aseptically
2. Excised leaf tissue exposed to callus inducing medium on agar plate
3. Callus induction — 3 – 5 weeks
4. Developing callus in agar medium (Propagation) — 2-4 months — 12 h light and dark cycle
5. Separation of Callus from agar
6. Gentle disintegration of callus tissue in to tiny fragments to obtain cells with intact cell wall
7. Visualization of cells under microscope
8. Transfer of cells into liquid medium
9. Separation of cells by centrifugation — Incubation at 25 °C to 30 °C — 2 weeks
10. Weighing of cell pellet
11. Snap freeze to disrupt cell wall
12. 1 g/ml solution in ethanol : water (70:30)
13. Crude hydro alcoholic extract of plant stem cells. Contains phytonutrients polyphenols, phycocyaninsanthocyanins, antioxidants
14. HPLC/MS Characterization
15. Lyophilization/High pressure homogenization
16. Powdered plant stem cell extract
17. Plant stem cell Microemulsion — Nanoparticle/Microemulsion — Formulated as serum

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Plant stem cell based products for hair growth

+ 8 Herbal Oils

Plant stem cell Microemulsion

PSC cream

Vehicle treated group

Animal 1 Hair growth score= 0

Animal 2 Hair growth score= 0

Animal 3 Hair growth score= 0
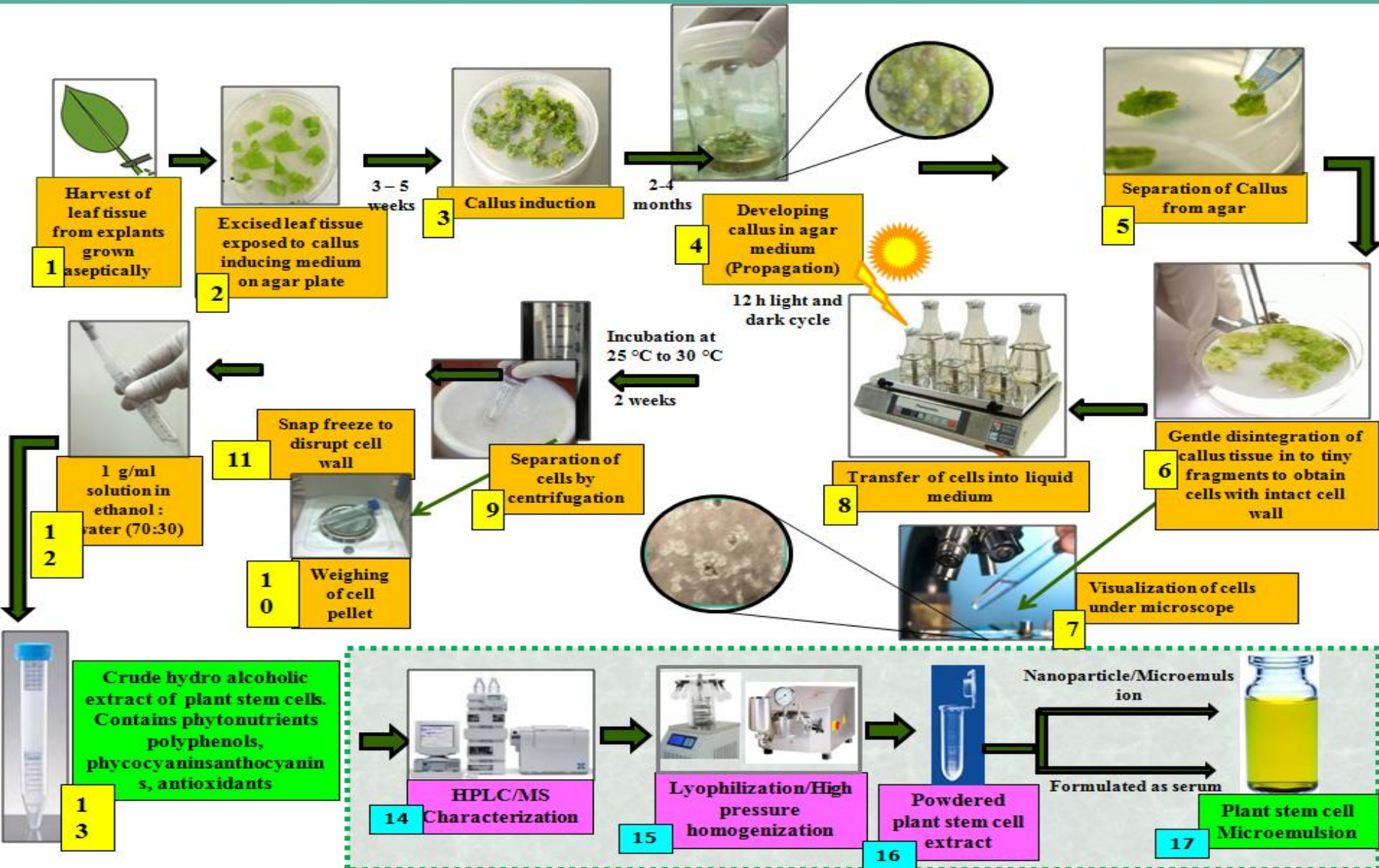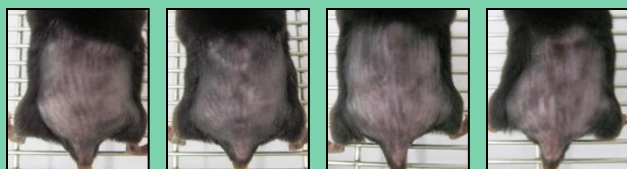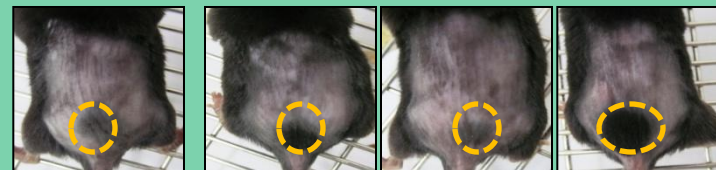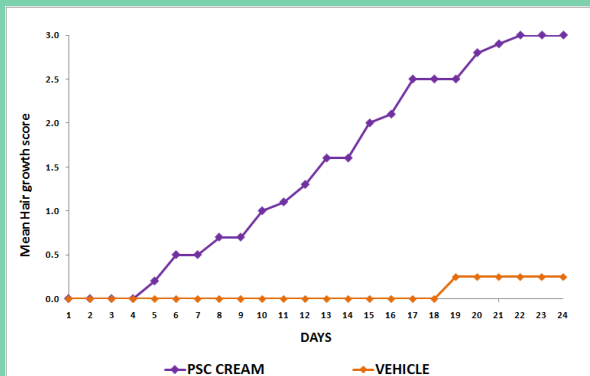
Animal 4 Hair growth score= 0

PSC treated group

Animal 1 Hair growth score= 2

Animal 2 Hair growth score= 3

Animal 3 Hair growth score= 0.5

Animal 4 Hair growth score= 2.5

Hair growth score

Group	Skin Color	% Anagen induction
PSC treated group	Black	100 % (4/4 animals)
Vehicle Control	Pink	0 % (0/4 animals)

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Hair growth Inhibition

❑ **Model**

- ❑ Animal models

❑**Test system**

- ❑ Swiss albino/C3H/HeJ mice

❑**Method**

- ❑ Application of test item to shaved skin
- ❑ Scoring of hair recovery

❑**End points**

- ❑ Hair growth retardation
- ❑ Weakening of hair shaft

Chemotherapy induced alopecia

❑ **Model**

- ❑ *In vivo* Model for screening molecules that prevent /reduce chemotherapy induced alopecia

❑**Test system**

- ❑ Swiss albino & C57BL/6 models

❑**Method**

- ❑ Synchronization of hair cycle to Anagen phase
- ❑ Induction of alopecia by Etoposide or Cyclophosphamide
- ❑ Testing of potential of molecule to prevent or reduce alopecia

❑**End points**

- ❑ Scoring for alopecia
- ❑ Hair weight

Hair pigmentation

❑ **Model**
- ❑ *In vitro* model

❑ **Test system**
- ❑ Murine B16 melanoma cells

❑ **Method**
- ❑ Determination of non-cytotoxic concentrations of test item
- ❑ Treatment with non-cytotoxic doses of test item
- ❑ Determination of the melanogenesis stimulation activity

❑ **End points**
- ❑ Melanin content
- ❑ Tyrosinase activity

❑ **Model**
- ❑ *In vivo* model

❑ **Test system**
- ❑ C3H/HeJ mice

❑ **Method**
- ❑ Administration of test item
- ❑ Hair color pigmentation effects

❑ **End points**
- ❑ Visual hair blackening
- ❑ Melanin content of hair

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Hair fiber damage and restoration

❑ **Model**

- ❑ Human hair samples

❑ **Method**

- ❑ Hair damage induced with dyes/chemical stress
- ❑ Application of test formulation
- ❑ Electron microscopic imaging

❑**End points**

- ❑ Ultrastructural visualization of hair damage and protection with test molecule
- ❑ Cuticular repair
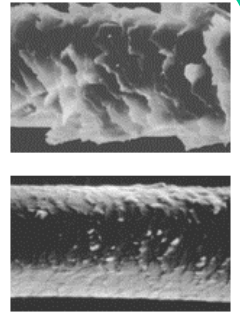
Conditioning, smoothing and softening properties

❑ **Model**

- ❑ Human hair samples

❑ **Method**

- ❑ Application of test formulation
- ❑ SEM imaging

❑**End points**

- ❑ Improvement of hair surface structure.
- ❑ Restoration of the slate-like hair scale arrangement.
- ❑ Absence of any lifted scales or any mechanical damage

Hair quality enhancers

❑ **Model**

- *In vivo* model

❑**Test system**

- C3H/HeJ or C57BL/6 mice

❑**Method**

- Administration of test item
- Analysis of plucked hair with visual and scanning electron microscopy

❑**End points**

- Hair shaft diameter
- Smoother hair cuticles
- Hair weight

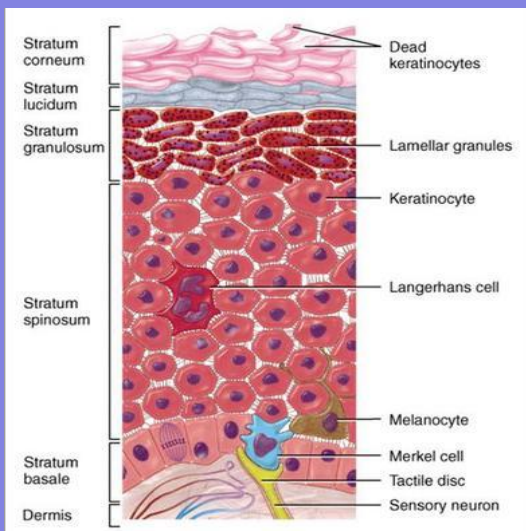## Photoprotection

❑ **Model**

- Human hair samples

❑**Method**

- Application of test formulation
- UV exposure
- SEM imaging
- Protein estimation

❑**End points**

- Ultrastructural visualization of hair damage and protection with test molecule
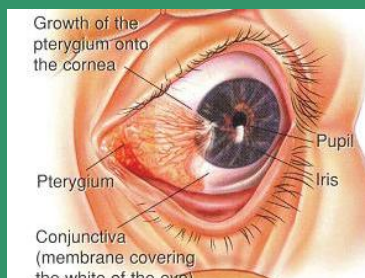- Hair protein content

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Skin irritation

- Keratinocytes

- Fibroblast cell line
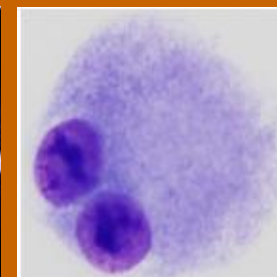
- 3-D reconstructed skin (OECD)

Occular irritation

HET-CAM assay *(invitox)*

Genotoxicity (OECD)

- Ames

- *In vitro* CA

- *In vivo* MNT

Solu...

THANKS

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