



A Study On Evaluation Of Hair Growth And Grey Hair Effect Of Poly Herbal Oil Formulation On Wistar Albino Rats

Manju Bhargavi N^{1*}, Pandya Devang J²

¹*Research Scholar, school of Pharmacy, RK University, mnidamanuri582@rku.ac.in

²Faculty of pharmacy, RK University, Rajkot, Gujarat, India, pandyadevang1982@gmail.com

*Corresponding Author: Manju Bhargavi N

*Research Scholar, school of Pharmacy, RK University, mnidamanuri582@rku.ac.in

(Received: 07 January 2024

Revised: 12 February 2024

Accepted: 06 March 2024)

KEYWORDS

Herbal oil, Hair growth, greyhair, ayurveda, ksheerapaka method etc.,

ABSTRACT

Hair has cosmetologically and sociological importance through out the life inframing the beauty and personality of an individual. Hair loss and hair graying at young age are recognized as a sign complex regulation of melanogenesis. Hair loss scientifically termed as alopecia and hair graying scientifically termed as canities. Now a days attention to herbal preparations have enormously increased due to least or zero side effects with desired results. In the present work selected herbs were used to study the effect of hair growth and the greying effect of hair. *Cocus Nucifera* oil is used as base oil along with *Moringa olifera* leaves, *Psidium guajava* leaves, *Carica papaya* leaves, *Trigonella foenumgraecum* seeds, *Allium cepa* bulb, and *Nigella sativa* seed oil. The *Salvia Rosmarinus* oil is used as essential oil. The herbal oil was prepared by using different methods and concentrations. The prepared formulations are tested by different evaluating methods, and the hair growth, skin irritation, hair length and grey hair test was done by using four sets of wistar rats. The effective hair growth was observed from day 2 and complete hair growth was recorded by 20 days after using of prepared hair oil applying topically on shaved wistar rats and grey hair effect was tested by using sheep wool fibers.

INTRODUCTION

On the scalp, where hair is usually densest and longest, the average total number of hairs is between 100,000 and 150,000. Human hair grows at a rate of about 0.5 inch (13 mm) per month. The absence of melanocytes, results in a loss of melanin pigmentation. Conditions such as albinism and phenylketonuria are caused by reduced or absent synthesis of melanin by melanocytes⁽¹⁾. Now a days hair fall is a very common issue faced by all the individuals. The reasons behind hair loss are due to aging, changes in hormones levels, medication, hereditary, etc., Although there are number of synthetic cosmetic products available in the market, the results after using these products were not to the expected mark⁽²⁾. Massaging of scalp with herbal hair oils play a great role in regrowth of lost hair. Not only hair re-growth but also thickening of hair follicles are additional advantage upon using herbal hair oils⁽³⁾. Haircoloris a kind of melanin pigment is deposited in hair shaft as it grows, but this hair-coloring process breaks down with age which is why grey hair is associated with advanced years⁽⁴⁾. According to Ayurveda excesspittadosha is a

common cause of premature grey hair. If hair fall is also associated, then vata is also involved as a main cause. In Ayurveda this condition is called pālitya or akāla palita (prematur e greying of hair). Pitta-provoking habits such as, anger and physical strain results in greying of hair. Intake of spicy, oily, salty andsour, fermented foods along with meat can aggravate Pitta⁽⁵⁾. Herbal hair oils are excellent substitutes to commercial hair dyes. Now in this present study the herbal hair oil was prepared by different herbs and oils and evaluated by various methods.

MATERIALS AND METHODS

There are various traditional methods was available for the preparation of hair oils. In this Direct boiling, Maceration, Water extraction and Kshreerapaka methods were followed to prepare different formulations by using different concentrations of herbs.

Collection of Plant Materials:

Fresh herbs were collected from RK University, Rajkot and authenticated process was completed for all herbal parts. The required oils were purchased from local



market in Ahmedabad. *Moringa olifera* leaves, *Psidium guajava* leaves, *Carica papaya* leaves, *Trigonella foenum-graecum* seeds, and, *Allium cepa* bulb, were collected in equal proportions. Drying of fresh herbs was carried out in a clean and dry shade. The dried herbs were finely powdered and stored in an air tight

container. Coconut oil, kalonji oil used as a base and rosemary oil was an essential oil. The chemical constituents and uses of all herbs and oils was given in Table no.1. **Procedure for Preparation of Hair Oil:** The herbal hair oil was prepared by using different herbs with different concentrations and methods. Table no.2

Table No. 2 Formulation of Herbal Hair Oil

Herbs used	D.B F1 (10)	M F2(10)	W. E F3(10)	KSH F4(10)	D.B F1(20)	M F2(20)	W. E F3(20)	KSH F4(20)
Methi seeds	10 gm	10 gm	10 gm	10 gm	20 gm	20 gm	20 gm	20 gm
Papaya leaves	10 gm	10 gm	10 gm	10 gm	20 gm	20 gm	20 gm	20 gm
Onion bulb	10 gm	10 gm	10 gm	10 gm	20 gm	20 gm	20 gm	20 gm
Moringa Leaves	10 gm	10 gm	10 gm	10 gm	20 gm	20 gm	20 gm	20 gm
Guava leaves	10 gm	10 gm	10 gm	10 gm	20 gm	20 gm	20 gm	20 gm
Coconut oil (or) CM	70 ml	70 ml	70 ml	CM (required quantity)	20 gm	20 gm	20 gm	CM (required quantity)
Kalonji oil	30 ml	30 ml	30 ml	30 ml	20 gm	20 gm	20 gm	30 ml
Rosemary oil	E. oil	E. oil	E. oil	E. oil	20 gm	20 gm	20 gm	E. oil

D.B: Direct boiling method, M: Maceration, W.E: Water extraction method, KSH: Ksheerapaka method, CM: Coconut milk

Table No.1 List of Herbs and oils with their Uses

S. No	Herb name and part	Biological Name	Chemical Constituent	uses
1.	Guava leaves	<i>Psidium guajava</i>	vitamin C and B lycopene, carotenoids, polyphenols, flavonoids & tannins	Treat dandruff, nourishes your hair roots, making them stronger. Hair growth. Protects your hair from the harmful ultraviolet rays of the sun preventing hair damage.
2.	Fenugreek seed	<i>Trigonella foenum-graecum</i>	Alkaloids, flavanoids and glycosides	Hair growth stimulant. Helps to make shine and black hair.
3.	Onion juice	<i>Allium cepa</i>	Sulfur	Promotes hair growth, prevent hair loss, strengthening hair, Prevent greying of hair
4.	Papaya leaf juice	<i>Carica papaya</i>	vitamin C, A, E and B, Minerals like magnesium and potassium. enzymes like papain and chymopapain	Increase volume to hair, gives natural shine increase hair growth and preventing baldness. Also, it prevents premature greying of hair. natural hair conditioner
5.	Moringa leaf	<i>Moringa oleifera</i>	vitamins, flavonoids, polyphenols, phenolic acids, alkaloids, tannins saponins, zinc iron, potassium, antioxidants	Rich anti-oxidants that can reduce Strengthens your hair, moisturizes scalp, promotes hair growth, helps hair retain its color.



6.	Coconut oil	<i>Cocos nucifera</i>	Catalase, peroxidase, amino acid vitamin C and biotin, triglyceride of lauric acid (fatty acid).	Treat hair fall, encourage regrowth, and prevent premature graying, nourish your hair, strengthen your roots, increase hair thickness and reduce hair loss, prevent premature graying of hair good hair conditioner
7.	Kalonji oil	<i>Nigella sativa</i>	vitamins, cymene, limonene, carvone, thymoquinone. Linoleic acid oleic, (23.4%) palmitic acid	fight hair falls and even to induce hair regrowth combating dandruff and scalp irritation. It prevents and even reverse graying of hair by preserving pigmentation in hair follicles
8.	Rosemary oil	<i>Salvia Rosmarinus</i>	1,8-cineol, camphor, α pinene, α -terpineolcamphene, borneol, limonene and p-cymene	Improve both hair thickness and hair growth, improve cellular generation, promote nerve growth.

valuation of Herbal Hair Oil:

The formulated herbal oil was evaluated for parameters like pH, acid value, saponification value, refractive index, viscosity and organoleptic parameters

Table 3: Evaluation Parameters f Herbal Hair Oil

S.no	Parameters	D.B F1/20	M F2/20	W.E F3/20	KSH F4/20	D.B F1/10	M F2/10	W.E F3/10	KSH F4/10
1.	REFRACTIVE INDEX at 20°C	1.456	1.4585	1.4565	1.458	1.452	1.4514	1.4647	1.4624
2.	Density	0.90501	0.90583	0.88669	0.89236	0.90587	0.90624	0.89051	0.90014
3.	Iodine value	66	79	83	96	71	95	89	102
4.	Peroxide value	0.91	1.49	2.98	3.74	0.95	1.82	3.25	4.11
5.	Saponification value	291	288	302	265	297	272	302	288
6.	LOD (%Loss on drying)	0.55	0.62	0.42	0.55	0.32	0.81	0.42	0.48
7.	Volatile oil content (%v/v)	0.24	0.41	0.35	0.41	0.14	0.17	0.18	0.25

Table 4: Organoleptic Property of Herbal Hair Oil

s.no	Parameters	D.B F1/20	M F2/20	W.E F3/20	KSH F4/20	D.B F1/10	M F2/10	W.E F3/10	KSH F4/10
1	Color	Pale greenish	Dark greenish	Pale brownish	Pale yellowish to greenish brown	Light greenish	Greenish to green	Pale light brownish	Pale greenish brown
2	smell	Traditional preparation smell with little burning smell also	Traditional preparation smells dominating coconut oil smell	Traditional preparation smell	Traditional preparation smell with milky odour	Traditional preparation smell with little burning smell also	Traditional preparation smells dominating coconut oil smell	Traditional preparation smell	Traditional preparation smell with milky odour

Animal Husbandry and Maintenance Adult 12 wistar strain albino rats (5–8 weeks, both male and female)

weighting 150-180 gm were placed in polypropylene cages with free access to standard laboratory diet and provided municipal water ad libitum. Each individual



animal was clinically examined and identified by fur marked with picric acid. The females were nulliparous and not pregnant at the time of experiment. Animals were grouped and housed in an environmentally controlled room with temperature of $22^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and 40-70% relative humidity with a 12-hour light-dark cycle and ventilation of 15-21 air changes/h for an acclimatization period of 7 days to laboratory conditions prior to the beginning of the experiment in order to adjust to the new environment and to overcome stresses incurred during their transit. Only healthy animals were assigned for these studies. The experimental protocol was approved by the Institutional animal ethics committee, Guuram Balanarasaiah Institute of Pharmacy, RR district, Telangana Approval number: IAEC/015/2022

Acute Dermal Irritation Study of Herbal Oil:

Male New Zealand White Rabbits were used for dermal irritancy experiment. The experimental protocol was approved by the Institutional animal ethics committee, Approval No. IAEC/014/2022, OECD 404 guidelines were adhered during the maintenance and experiment. In dermal irritancy study F420 formulation was used on rabbits ⁽⁶⁾. Approximately 24 hours before the test, fur was removed by closely clipping the dorsal area of the trunk of the animals. 1 ml herbal oil formulation was applied to a small area (approximately 6 cm²) of skin and covered with a gauze patch, which is held in place with non-irritating tape. All animals were examined for signs of erythema and oedema, and the responses scored at 60 minutes, and then at 24, 48 and 72 hours after patch removal ⁽⁷⁾.

Treatment for Hair Growth Activity in Vitro Adult 12 wistar strain albino rats were divided into four groups of 3 animals in each group. Hairs from 3 cm² area at the dorsal portion of all the rats were shaved using electric shavers and applied with marketed hair remover to completely remove hair. The animals were divided into 4 groups, 3 animals in each group. Group I was kept as control, group II was studied with standard drug 2% of minoxidil and rest four group was treated with herbal oil formulation i.e. F420 1ml and F420 2 ml. The standard

Results Table 5: Acute Dermal Irritation Study

Skin irritation	0.8% formalin			F420 1ml			F420 2ml		
	24 hr.	48 hr.	72 hr.	24 hr.	48 hr.	72 hr.	24 hr.	48 hr.	72 hr.
Observation time									
Total score	18	13	11	0	0	0	0	0	0
Mean score	6.11	5.21	7.33	0	0	0	0	0	0
Total of mean score	31.76			0			0		
PII	10.58			0			0		
Remarks	Severely irritant			Non irritant			Non irritant		

drug minoxidil solution (2%) was applied on the rats as positive control.

Qualitative Study of Hair Regeneration

Qualitative hair growth was evaluated by observation of two parameters: hair growth initiation time and hair growth completion time i.e., minimum time taken to cover the denuded skin region with new hair completely. Hair growth initiation and completion time were recorded for each group of animals and compared with the positive control minoxidil 2% solution and control. Also, the average length and weight of hair was recorded and compared for each group of animals ⁽⁸⁾.

Application of the Herbal Hair oil on Bleached Sheep Wool Fibers for Grey Hair Test

The natural bleached sheep wool fibers were collected from local market, Hyderabad, India. The wool fibers were cut into small pieces (15 cm). Each wool fibers defatted with Petroleum ether (40°C – 60°C). The five wool pieces were dipped into each formulation kept in a beaker for 1 h, air-dried and washed with tape water. The washed colored wool fibers were divided into four categories to observe the effects at room temperature, in sunlight and washing with a natural detergent. ⁽⁹⁾

Effect of Room Temperature on Colored Wool Fibers

The colored wool fibers were pasted on a white paper sheet, covered with transparent cellophane sheet and then kept for 30 days at room temperature.

Effect of Sunlight on Colored Wool Fibers

The colored wool fibers were pasted on a white paper sheet, covered with transparent cellophane sheet and kept in day sunlight for 2 h daily for 30 days.

Effect of Natural Detergent on Colored of Wool Fibers

The colored wool fibers were washed with aqueous reetha (soap nut) extract for 5 min on alternate days for 30 days and then air-dried after each washing.

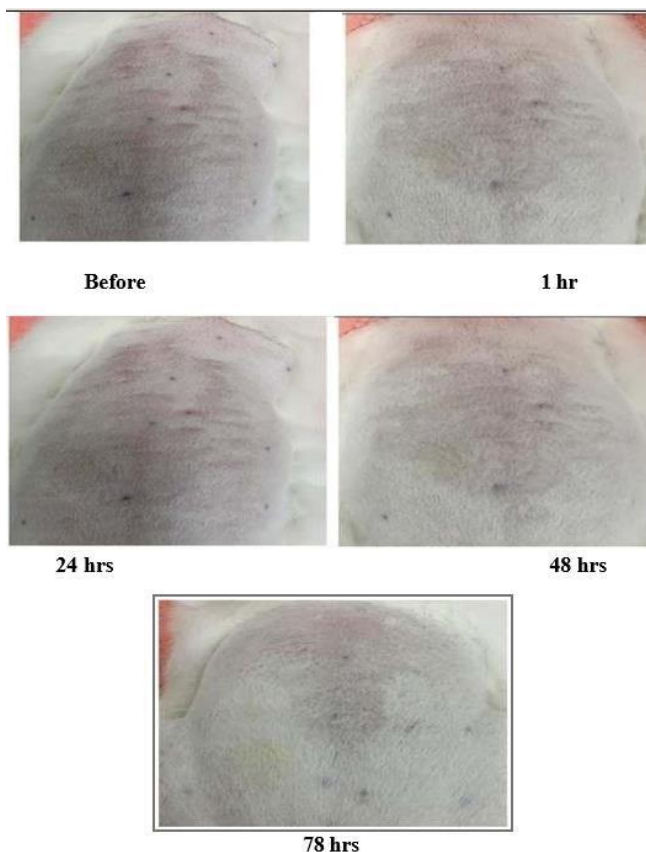
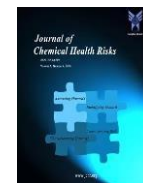


Figure 1: Acute dermal irritation study images

Hair growth

Table 6: Density of Hair Follicles

Treatment	Hair Growth Days		
	7days Mean ± SD	10days Mean ± SD	15days Mean ± SD
Minoxidil	8±1.69	21±0.82	29±0.31
F420 1ml	9±2.13	20±0.66	31±0.11
F420 2ml	14±0.76	27±0.43	34±0.26

(N.B. The values presented here are average of three repetitions of study)

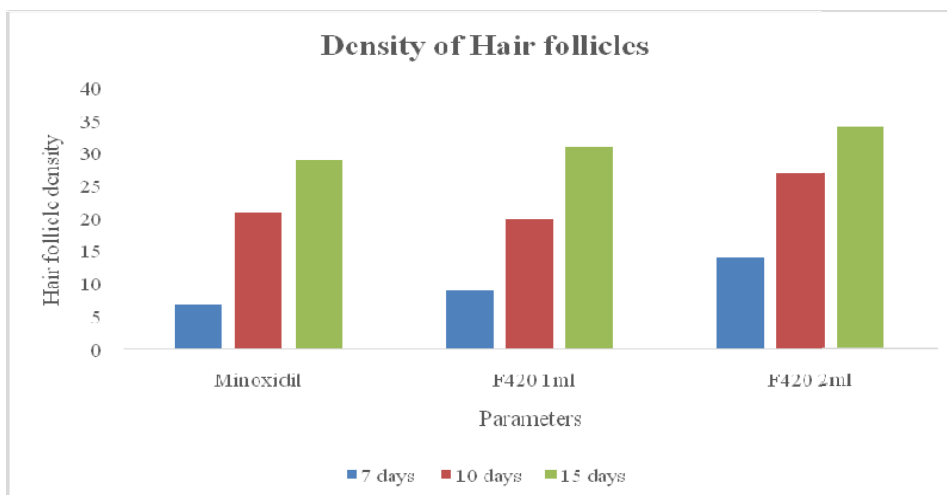
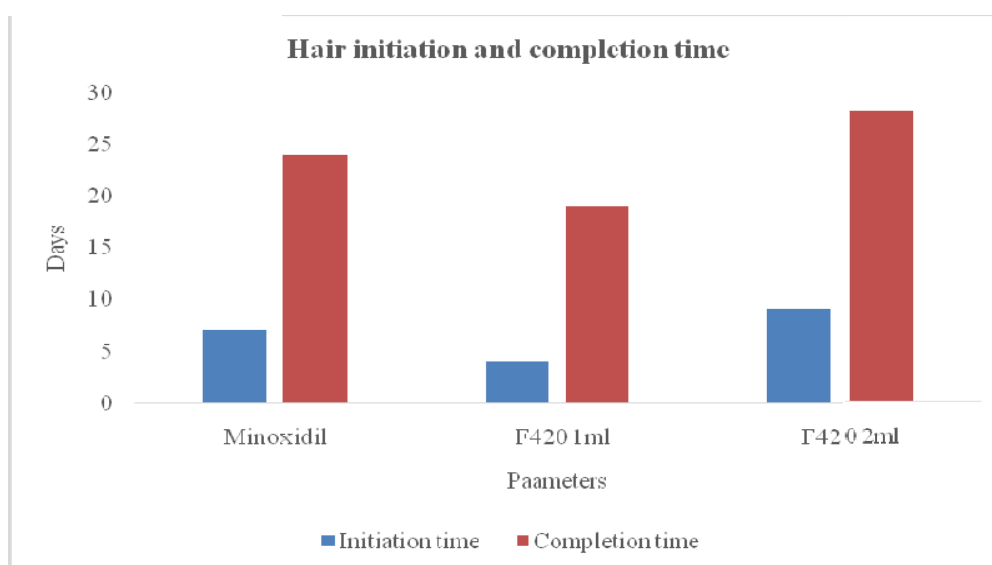


Figure 2: Graphical representation of density of hair follicles

**Table 7: Hair Growth Initiation and Completion Time**

Treatment	Hair Growth Days	
	Initiation time(days) Mean \pm SD	Completion time (days) Mean \pm SD
Minoxidil	7 \pm 0.58	24 \pm 0.62
F420 1ml	4 \pm 0.82	19 \pm 0.51
F420 2ml	9 \pm 0.45	28 \pm 0.35

(N.B. The values present adhere are average of three repetitions of study)

**Figure 3: Graphical Representation of Hair Initiation and Completion Time****Table 8: Length and Weight of Hair Intreated Groups**

Treatment	Length of Hair(cm) Mean \pm SD	Rate of elongation (cm/day) Mean \pm SD	Weight of hair(mg)	Rate of increment (mg/day) Mean \pm SD
Minoxidil	2.7 \pm 0.37	0.11	4.3 \pm 0.97	0.18
F420 1ml	2.9 \pm 0.46	0.12	4.1 \pm 1.04	0.17
F420 2ml	3.1 \pm 0.84	0.16	5.3 \pm 1.12	0.27

(N.B. The values presented here are average of three repetitions of study)

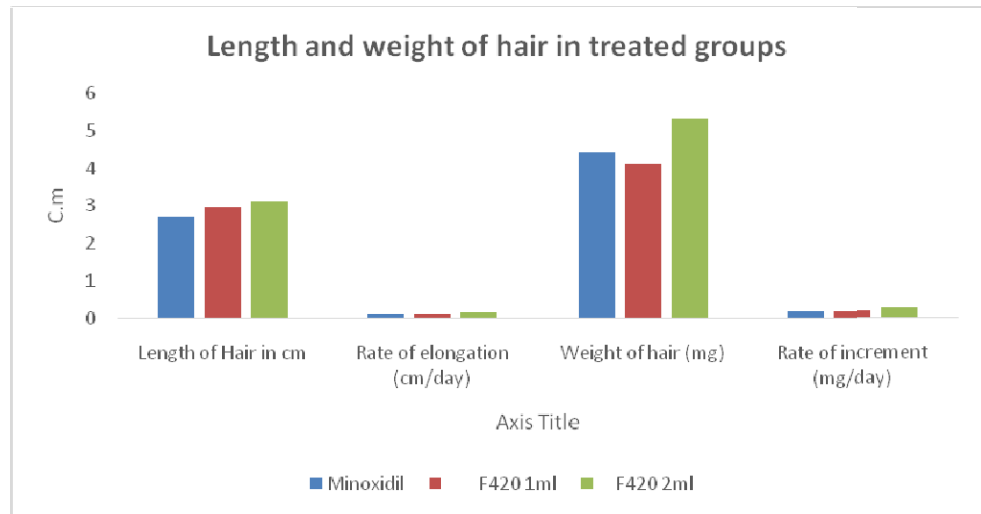


Figure 4: Graphical Representation of Length and Weight of Hair in Treated Groups



Figure.5. a) Control group wistar rats without any treatment



Figure.5. b) Hair regeneration in wistar rats treated with 2% Minoxidil solution



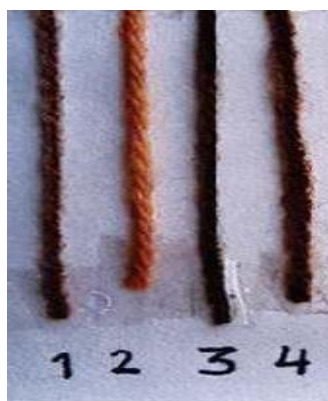
Figure.5.c)Hair Regeneration in Wistar Rats Treated with420 1 ml



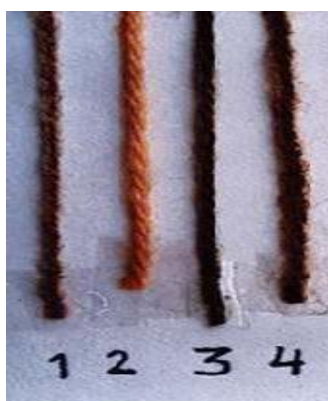
Figure.5. d) Hair regeneration in wistar rats treated with F420 2 ml

Table 9: Grey Hair Test of Hair oil using with Sheep Wool Fibers.

S.No	Parameters	1(Market Product		Based2(control)		3(F4 20 /1 ml)		4(F4 20 /2ml)	
		15 days	30 days	15days	30 days	15 days	30 days	15 days	30 days
1.	Effect of room temperature	No shading	Slightly changed	Color changed	Color changed	No shading	No shading	No shading	No shading
2.	Effect of sunlight	Slightly changed	Color changed	Color changed	Color changed	No shading	Slightly changed	No shading	No shading
3.	-----	Shading of Color		-----	Color changed	-----	Slightly changed	-----	Slightly changed



Effect of room temperature of colored sheep wool threads stained with herbal oil 15th day



Effect of sunlight of colored sheep wool threads stained with herbal oil 15th day



Effect of Sunlight of Colored Sheep wool threads stained with herbal oil 30th day

DISCUSSION

The acute dermal irritation study, skin irritation was performed on Male New Zealand White Rabbits with 0.8% formalin, F420 1ml, F420 2ml for 24 hr., 48 hr., 72 hrs. by the application of 0.8% formalin shows total mean score of 31.76 and the herbal formation F420 with 1 ml, 2 ml shows 0 irritation score. In the rats, the initiation of hair follicles was observed in the F 420 1ml treated wistar rats on 4 ± 0.82 days and the completion of hair growth was observed on 19 ± 0.51 days. However, the initiation of hair follicles took more time in the case of F 420 2ml and minoxidil treated wistar rats i.e., 9 ± 0.45 and the complete hair growth was observed on 28

± 0.35 . The quality of hair was soft and silky in the case of F 420 2ml and minoxidil treatments. The average length of the minoxidil treated wistar rats hairs was 1.92 ± 0.37 cm and the average weight 4.3 ± 0.97 mg, for 10 numbers of hairs collected from the group. In the case of F 420 1ml and F 420 2ml treated group, the average length of hairs was 1.77 ± 0.46 and 1.58 ± 0.84 with average weight 4.1 ± 1.04 mg 5.3 ± 1.12 and mg respectively for 10 numbers of hairs collected from each group. Visual observation on hair growth was recorded on 13th day with minoxidil treated positive control group. Hair growth was visually observed on 7th day after 420 1ml treatment whereas F 420 ml. treated wistar rats showed hair growth on 9th day. The complete



hair growth was observed on 21st day in the case of F 420 1ml and 24th day in the case of F 420 2ml compounds. But in the case of minoxidil, complete hair growth was observed on 25th day following the treatment. Regeneration of hair intreated with minoxidil and herbal oil are showed in Fig.5 a-c and the data obtained from the study are presented in Table 8.

There was no change in color of wool fibers when kept at room temperature for 10 days, whereas the color of wool fibers got faded in sunlight gradually and after a period of 15 days it remained half of the original color. It indicated that ultraviolet rays present in sunlight affected the color of wool fibers produced by herbal hair oil formulation. The washing of wool fibers with reetha extract (a natural detergent) on alternate days did not affect quickly on colored fibers.

The maximum coloring effect was observed with F4. The colorant and color on wool fibers after treatment with F4 remained for the maximum days, when these kept at room temperature, in sun light and washed with the natural detergent. The intensities of color on these fibers by herbal hair oil formulation were observed in the order: 4 >3>1>2.

CONCLUSION

Based the above results by comparing with standards, the herbal formulations F420 at 2 ml shows zero skin irritation, effective hair initiation, growth and hair weight.

REFERENCES

1. Britannica Publisher: Encyclopaedia Britannica, Inc: melanocyte, Encyclopaedia. Date Published:
2. Mayo Foundation for Medical Education and Research 2 March 26, 2022, <https://www.mayoclinic.org/diseasesconditions/hair-loss/symptoms-causes/syc20372926>.
3. Lopa K: Essential oils for hair growth and health. Nov 08, 2022, <https://www.bebeautiful.in/allthings-hair/everyday/9-essential-oils-for-hair-growth-and-health>.
4. Medline plus: U.S department of health and human services, National institution of health, Rockville pike, Bethesda, MD20894: July 8, 2022.
5. Srinivas Krishnaswamy: hair, skin & homecare products krya botanicals research & manufacturing. Siruseri, Tamilnadu: May 25, 2023: goodies@krya.in.
6. Wang J, Li Z, Sun F, Tang S, Zhang S, Lv P, Li J, Cao X. Evaluation of dermal irritation and skin sensitization due to vitacoxib. *Toxicol Rep.* 2017 Jun 10; 4:287-290.
7. Djerrou Z, Djaalab H, Riachi F, Serakta M, Chettoum A, Maameri Z, Boutobza B, Hamdi-Pacha Y. Irritantcy potential and sub-acute dermal toxicity study of Pistacia lentiscus fatty oil as a topical traditional remedy. *Afr J Tradit Complement Altern Med.* 2013 Apr 12;10(3):480-9
8. Patel, S., Nag, M.K., Sharma, V., Chauhan, N.S. and Dixit, V.K., 2014. A comparative in vivo and in vitro evaluation of hair growth potential of extracts and an isolate from petroleum ether extract of *Cuscuta reflexa* Roxb. *Beni-Suef University Journal of Basic and Applied Sciences*, 3(3), pp.165-171.
9. Patel, S., Nag, M.K., Sharma, V., Chauhan, N.S. and Dixit, V.K., 2014. A comparative in vivo and in vitro evaluation of hair growth potential of extracts and an isolate from petroleum ether extract of *Cuscuta reflexa* Roxb. *Beni-Suef University Journal of Basic and Applied Sciences*, 3(3), pp.165-171.