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Pharmaceutico-analytical, acute dermal toxicity and invitro Efficacy of *Keshakrishneekarana Lepa*

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ABSTRACT

Premature greying of hair, described in *Ayurveda* as *Palithya*, has become increasingly common, particularly due to stress, genetics, and nutritional deficiencies. Conventional hair dyes may cause scalp irritation, hair damage, and other adverse effects. The present study was undertaken to prepare *Keshakrishneekarana Lepa* and evaluate its pharmaceutico-analytical profile, acute dermal toxicity, and in vitro hair-darkening efficacy on human grey hair. The formulation was prepared according to the classical reference in *Arka Prakasha* using *Triphala*, *Nili Patra*, *Bhringaraja*, *Ayomala* (*Mandura*), and *Avimutra Arka*, and it was then subjected to analytical evaluation. Acute dermal toxicity was assessed on Wistar albino rats as per OECD guideline 402. In vitro efficacy was studied on human grey hair strands by repeated topical application of the formulation and observation of colour change. The formulation showed acceptable physicochemical properties. In the animal study, the formulation showed no irritation, behavioural alterations, or mortality. The primary dermal irritation index was 0.0, and gross necropsy revealed no pathological changes. In the in vitro study, the grey hair strands showed a gradual and progressive darkening with repeated application, and by the 7th application most strands had attained dark brown or black colouration, although a few grey strands still persisted. Overall, *Keshakrishneekarana Lepa* was found to be safe for dermal application under the study conditions and demonstrated promising in vitro hair-darkening activity. These findings suggest its potential as an *Ayurvedic* alternative to synthetic hair dyes, although further clinical studies are required to establish its therapeutic utility.

Key Words Acute dermal toxicity, *Arka Prakasha*, in vitro hair-darkening, *Keshakrishneekarana Lepa*, *Palithya*

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INTRODUCTION

Ayurveda refers greying of hair as *Palithya* or *Palitha*, a condition linked to an imbalance in the *Pitta dosha*¹. Acharya Sharangadhara lists *Akaala Palitha* (Premature greying of hair) as a feature of *Pitta Prakruthi*². In the present era, early greying is seen even in young adults, and is often associated with stress, genetics, nutritional deficiencies, and altered lifestyle practices. As a result, hair dyeing has

become a commonly adopted measure among affected individuals.

Hair dyes available in the market contain various ingredients including primary dyeing agents like Paraphenylenediamine (PPD), Ammonia for opening the hair cuticle, Hydrogen peroxide as the developer, and various conditioning agents. While these products are effective cosmetically, they are associated with adverse effects such as allergic reactions, scalp irritation, and hair damage like dryness, breakage, and

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loss of hair elasticity. Some studies suggest potential links between certain hair dye ingredients and health risks like cancer.

Safer hair dyes are formulated with gentler ingredients that minimize the risk of adverse effects while still providing effective colour coverage. While these dyes may require more frequent application or have limitations in colour range compared to traditional dyes, they offer a preferable option for those prioritizing safety and hair health.

Ayurveda has various dosage forms for drug administration. *Acharya Ravana* has mentioned that *Arka* preparations are the most effective form as these are *Dosharahitha* and *Gunasanghaprakasham*³. In *Arka Prakasha*, the author has mentioned *Keshakrishneekarana Lepa* can be applied to obtain *Krishna varna* of the hair⁴.

Many herbal hair dyes are available in the market in the form of pastes and powders. However, the use of *Arka* in such formulation has not been reported to date. Therefore, an attempt was made to formulate a hair dye utilizing non-toxic ingredients and to study the acute dermal toxicity and in vitro efficacy of *Keshakrishneekarana Lepa*.

MATERIALS AND METHODS

Study design

This experimental study had three components: pharmaceutical preparation of *Keshakrishneekarana Lepa*, and its analytical and microbiological evaluation, assessment of its acute dermal toxicity and in vitro hair-darkening activity.

Drug procurement and authentication

Ingredients of *Keshakrishneekarana Lepa* were procured from Amruth Kesari Depot, Bengaluru, while fresh plant materials were collected from the

herbal garden of the Department of *Dravyaguna*. Fresh *Avimutra* was obtained from farms located in Doddaballapur, Bengaluru. All raw materials were identified and authenticated by experts from the Department of *Rasashastra* and *Bhaishajya Kalpana* and the Department of *Dravyaguna* at Sri Sri College of Ayurvedic Science and Research, Bengaluru, and the relevant details were systematically documented.

Preparation of the test formulation

Keshakrishneekarana Lepa was prepared according to the classical textual references at the Department of *Rasashastra* and *Bhaishajya Kalpana*, Sri Sri College of Ayurvedic Science and Research, Bengaluru.

Dried fruits of *Amalaki* (*Embllica officinalis* Gaertn.), *Haritaki* (*Terminalia chebula* Retz.), and *Bibhitaki* (*Terminalia belerica* Roxb.), 500g each, were powdered and passed through mesh no. 85 to give *Churna*. Fresh *Nili* (*Indigofera tinctoria* Linn.) leaves and *Bhringaraja* (*Wedelia calandulacea* Less.) whole plant was shade-dried for five days and similarly reduced to fine powder.

Ashuddha Mandura (500g) was subjected to *Shodhana* by the *Nirvapa* method, in which pieces were heated to red hot (630-670°C) and quenched in fresh *Gomutra* for seven cycles⁵, yielding *Shuddha Mandura*. This was triturated with *Kumari Swarasa*, made into *Chakrikas*, and subjected to *Marana*⁶ in an electric muffle furnace across 17 *Putra* cycles, with gradual increasing temperature of 500-700°C as per *Parishishta of Rasamritam*⁷.

Avimutra (sheep urine) was filtered and distilled, discarding the initial fraction, to obtain *Avimutra Arka* at approximately 60% recovery of the starting volume⁸.

Equal quantities (50g each) of the six components - *Amalaki*, *Haritaki*, and *Bibhitaki Churna*, *Nili patra Churna*, *Bhringaraja Churna*, and *Mandura*

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Bhasma were mixed thoroughly and sieved through mesh no. 85 to give *Lepa Churna*. For each application, the powder was freshly mixed with *Avimutra Arka* to form the *Lepa*⁴.

Analytical evaluation

The analytical characteristics of the *Lepa* formulation were evaluated in the laboratory of Sri Sri Tattva, Bengaluru.

Acute dermal toxicity study

The study followed the limit-dose procedure of OECD Test Guideline 402 (Fixed Dose Procedure, 2017)⁹, with ethical clearance from the Institutional Animal Ethics Committee (IAEC), Department of Pharmacology, Acharya & B.M. Reddy College of Pharmacy, Bengaluru (IAEC/AMRCP/PR/2025-2026/40), under CPCSEA norms. Healthy, nulliparous, non-pregnant female Wistar albino rats weighing 150-200 g were acclimatized for five days, examined for baseline body weight, skin integrity, and behaviour, and then randomly assigned by weight into two groups of three animals each. The control group received distilled-water-moistened gauze, while the trial group received *Keshakrishneekarana Lepa* at the OECD limit dose of 2000 mg/kg, applied topically over clipped dorsal skin covering about 10% body surface area and secured with dressing, as shown in Figure 1 and 2.

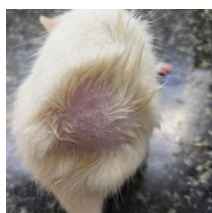


Figure 1 Hair removed on the dorsal surface



Figure 2 Application of test formulation

Animals were observed for clinical signs at 30-60 minutes post-dose, hourly on day 1, and daily thereafter for 14 days, with dermal irritation scored on the Draize scale (0-4) for erythema and oedema. Body weight was recorded on days 0, 7, and 14. Necropsy was performed on day 15, with external and internal examination of organs and the application site. Body weight data were tested for normality (Shapiro-Wilk) and compared between groups by unpaired Student's t-test using R software, with $p < 0.05$ taken as significant.

In vitro efficacy study

Grey or white hair strands were collected from healthy adult volunteers, excluding any strand with a history of chemical treatment, dye, or henna use, or visible structural damage. Strands were rinsed with distilled water, air-dried, and arranged in sterile petri dishes. Baseline colour was recorded on a five-point ordinal scale developed for this study: 0 (grey/white), 1 (grey with faint light-brown tinge), 2 (light to medium brown), 3 (dark brown), and 4 (black), as shown in Figure 3.



Figure 3 Hair colour scale illustrating representative shades used for scoring.

For each application cycle, 10 g of freshly prepared *Lepa* (*Lepa Churna* mixed with *Avimutra Arka*) was applied uniformly to the strands, left undisturbed for 60 minutes at room temperature, then rinsed gently with distilled water for about 60 seconds without mechanical rubbing, air-dried, and rescored. This cycle of application, contact, rinsing, drying, and scoring was repeated on the same strands until a score of 4 was reached or no further darkening occurred. The primary outcomes were the colour score at each

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cycle and the number of applications needed to reach score 4; the secondary outcome was whether all strands ultimately reached score 4.

RESULTS

Pharmaceutical preparation

Each stage of preparation proceeded as per the classical reference. *Shodhana* of *Mandura* by *Nirvapa* reduced 500 g of *AshuddhaMandura* to 443.6g of *Shuddha Mandura* over seven cycles, with heating time falling from 45 minutes in the first cycle to 15 minutes in the seventh as the material became progressively more brittle. *Maranath* through 17 *Putra* cycles yielded *Mandura Bhasma* weighing 417g, which satisfied all classical *Bhasma Pariksha* (*Rekha* *purna*, *Slakshna*, *Varitaratva*, *Unnamana*, *Apunarbhava*, and *Niruttha* tests) and passed completely through mesh no. 200.

Avimutra Arka distillation gave 460 mL of clear, colourless distillate from 750mL of starting material. The final *Lepa Churna*, blended from the six standardized components, was a uniform, non-gritty green powder weighing 300 g.

Analytical parameters

A) Organoleptic Parameters:

- **Colour:** Uniform greenish brown
- **Odour:** Characteristic herbal odour
- **Taste:** Predominantly *Kashaya* with *Tiktha*
- **Touch:** Smooth, soft, non-gritty; fine powder consistency

B) Physicochemical Parameters:

Physicochemical and microbiological findings are summarised in Table 1.

Table 1 Physicochemical parameters of <i>Keshakrishneekarana Lepa</i>		
S.NO	TEST	RESULTS
1.	pH	7.68

2.	Loss on drying	72.19%
3.	Total ash	20.88%
4.	Acid insoluble ash	16.54%
5.	Water soluble ash	18.39%
Microbiology Test		
1.	<i>E. coli</i>	Absent
2.	<i>Salmonella spp.</i>	Absent
3.	<i>Staphylococcus aureus</i>	Absent
4.	<i>Shigella spp.</i>	Absent
5.	<i>Pseudomonas</i>	Absent
6.	Total Microbial Count	100cfu/g
7.	Total Yeast & Mold	<10cfu/g

Acute dermal toxicity

No mortality, lethargy, salivation, convulsions, or other behavioural change occurred in either group across the 14-day observation period. No erythema or oedema was recorded at any time point on the Draize scale, giving a Primary Dermal Irritation Index of 0.0 in both groups.

Body weight increased steadily in both groups over the study period, as shown in Table 2 and Figure 4, with no statistically significant difference between control and treatment groups at any time point ($p > 0.05$).

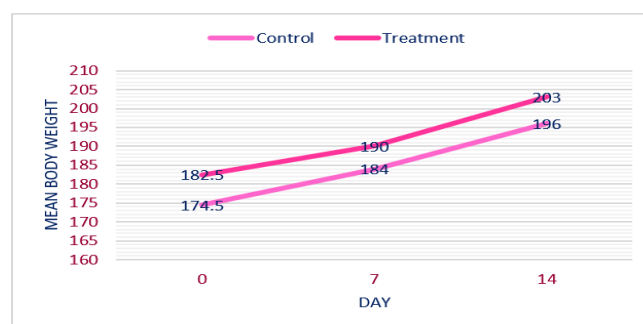


Figure 4 Mean body weight changes in control and treatment groups of Wistar albino rats over 14 days.

Table 2 Mean $\pm$ SD body weight (g) of control and treatment groups on days 0, 7 and 14 in the acute dermal toxicity study.

GROUP	DAY 0 (g)	DAY 7 (g)	DAY 14 (g)	% GAIN*
Control	174.5 $\pm$ 7.47	184.0 $\pm$ 7.00	196.0 $\pm$ 6.00	12.32092
Treatment	182.5 $\pm$ 5.07	190.0 $\pm$ 5.57	203.0 $\pm$ 5.29	11.23288

*: % GAIN = [(Day 14 bw – Day 0 bw) / Day 0 bw] $\times$ 100

Gross necropsy on day 15 revealed no pathological change in any organ or at the application site in either group. Since no adverse effect occurred at the limit

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dose of 2000 mg/kg, *Keshakrishneekarana Lepa* is classified as GHS Acute Dermal Toxicity Category 5, or unclassified, with an estimated acute toxic dose exceeding 2000 mg/kg.

In vitro efficacy study

Table 3 shows a cumulative darkening effect of *Keshakrishneekarana Lepa* on grey hair strands, with a progressive change from grey/white (score 0) to predominantly black (score 4) by the 7th application. The response was gradual rather than immediate, and slight strand-to-strand variation persisted through the final cycle, as shown in Figures 5,6,7 and 8.

Table 3 Progressive changes in hair colour score with repeated application of *Keshakrishneekarana Lepa* (in vitro)

APPLICATION NUMBER	HAIR COLOUR SCORE(0-4)	DOMINANT OBSERVED SHADE
0 (Baseline)	0	Grey/white
1	0.5	Grey with very faint light brown tinge
2	1.0	Light brown visible in some strands
3	2.0	Light to medium brown in majority of strands
4	2.5	Medium to dark brown
5	3.0	Dark brown
6	3.5	Very dark brown
7	4.0	Black in majority, some grey strands persisting

DISCUSSION

The pharmaceutico-analytical observations indicate that *Keshakrishneekarana Lepa* can be prepared consistently from its classical reference and that finished products meet the basic quality standards expected of a topical *Ayurvedic* formulation. Its pH, 7.68, is close to the normal scalp skin and may cause mild hair cuticle swelling, which could support pigment uptake during application without causing irritation. The comparatively high loss on drying

reflects the semisolid, moisture-retaining nature of a *Lepa* rather than a defect in stability. Ash values are mainly attributable to *Mandura Bhasma*, and their moderate values suggest that the *Shodhana* and *Marana* processes were effective in eliminating impurities. Despite the moisture content, no pathogenic organisms were detected, and the total microbial counts remained within acceptable pharmacopoeial limits, indicating good hygiene during preparation and handling.



Figure 5 Human grey hair strands



Figure 6 After 3rd application



Figure 7 After 5th application



Figure 8 After 7th application

Acute dermal toxicity was carried out because there was no published information on the dermal safety of *Avimutra Arka*, and its effects on skin. A limit test with three animals per group was chosen to obtain an initial safety rather than a full dose-response profile, while also keeping animal use to a minimum in line with reduction principles and OECD 402 guidelines. As no irritation, behavioural changes, or necropsy abnormalities were seen, the formulation

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was placed in GHS Category 5 (unclassified), which is the lowest hazard category.

The in vitro results showed a steady darkening of the grey hair strands over seven applications, which is similar to the gradual colour change reported with many herbal hair dyes. The variation in hair colour among strands may be due to natural differences in porosity and cuticle structure, which can affect pigment uptake even under in vitro conditions. Grey and white hairs are generally more resistant to colour penetration because of their compact cuticle, lower lipid content, and differences in melanin content, resulting in uneven dye absorption¹⁰. The findings should also be interpreted cautiously, as real-life factors such as heat, sweat, sebum, and environmental exposure were not fully replicated in the laboratory setting.

The exact mode of action of *Keshakrishneekarana Lepa* cannot be confirmed from this study alone, but a plausible explanation can be suggested from the properties of its ingredients. In *Ayurvedic* terms, the formulation appears predominantly *Pitta-Shamaka*, which is relevant in *Palithya*. From a modern perspective, it may work through gradual deposition of colouring compounds on the hair shaft with repeated application.

Triphala may contribute through its tannin content, which can bind to keratin and gradually darken the hair. *Amalaki* may also support antioxidant protection because of its vitamin C content, helping reduce oxidative damage linked to premature greying¹¹. Yellow *Bhringaraja* was selected for the formulation due to its availability, this choice is supported by classical commentary stating that *Swetha* and *Peeta Bhringaraja* share similar properties, including *Kesharanjana*¹², and by modern evidence showing that *Wedeliacalendulacea* has higher total phenolic

content¹³. *Bhringaraja* is traditionally considered beneficial for preserving natural hair colour and may help protect hair follicle cells from oxidative stress. *Bhringaraja* also stimulates the outer root sheath cells of the follicle, which supports hair growth, strength, and natural lustre¹⁴.

Nili is a known natural dye source and may darken hair by depositing indigotin pigments on the hair shaft¹⁵. *Mandura Bhasma* supplies iron, which is required for oxygen delivery to the follicle and may support melanin synthesis pathways. In this context, *Amalakimay* enhance iron bioavailability through ascorbic acid-mediated chelation, improving the uptake of iron from *Mandura Bhasma*¹⁶. *Avimutra Arka* may act as a penetration enhancer and bioenhancer, helping the active components reach the hair shaft and follicular area more effectively. Overall, the formulation may act through a combination of surface pigmentation, antioxidant support, follicular nourishment, and enhanced penetration of active constituents.

The limitations of this study are that the in vitro hair model did not fully replicate real scalp conditions, the acute dermal toxicity assessment was restricted to short-term exposure, and no repeated-dose or long-term safety evaluation was performed. In addition, practical challenges in the collection and distillation of *Avimutramay* affect scalability and routine preparation of the formulation.

CONCLUSION

In this study, *Keshakrishneekarana Lepa* was successfully prepared according to its classical reference and met the basic organoleptic, physicochemical, and microbiological standards for a topical *Ayurvedic* formulation. In the OECD 402

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acute dermal study at a limit dose of 2000 mg/kg, Wistar rats showed no irritation or signs of systemic toxicity, and the in vitro hair experiment demonstrated a gradual darkening of grey human hair strands with repeated use. These results together offer

preliminary support for its pharmaceutical quality, short-term dermal safety, and efficacy, and they help document a formulation that has not previously been described in detail in research literature.

ANIMAL STUDY – ETHICAL COMMITTEE CERTIFICATE

CCSEA Committee for the Control and Supervision of Experiments on Animals		 ACHARYA	
INSTITUTIONAL ANIMAL ETHICS COMMITTEE ACHARYA & BM REDDY COLLEGE OF PHARMACY Registration No: 997/PO/Re/S/06/CPCSEA			
Chairman Dr. Manjunatha PM		Ref.: IAEC/ABMRCP/PR/2025-2026/40	
Scientist from different Biological discipline Dr. Grubisavara Swamy Dr. Uday Raj Sharma		Date: 06/09/2025	
Veterinarian Dr. Ramesh BG		This is to certify that the dissertation proposal entitled	
Scientist in charge of AHF Dr. Suresh Janadri		“Pharmaceutico-analytical, acute dermal toxicity and invitro efficacy of Keshakrishneekarana Lepa” submitted by Dr. Shraveena. R. ,	
CPCSEA Nominees		student of Sri Sri College of Ayurvedic Science and Research, Bengaluru	
Main Nominee Dr. Krishnappa H		has been approved by IAEC.	
Socially Aware Member Mr. Ramanaiah Illuri			
Scientist From Outside the Institute Dr. Mohan Kumar Shettar			
Member Secretary Dr. Suresh Janadri		CPCSEA-Main Nominee Dr. Krishnappa H	
			
		Chairman-IAEC Dr. Manjunatha PM Institutional Animal Ethical Committee Acharya & B.M. Reddy College of Pharmacy Bangalore - 560 107	
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