Original Article

Evaluation of hair growth potential of *Angustifolia* leaves hair gel in rats

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ABSTRACT

Background: Hair loss, or alopecia, significantly affects both physical appearance and psychological well-being, particularly in cases such as androgenetic alopecia and alopecia areata. Current pharmacological treatments like minoxidil and finasteride have limitations, including side effects and decreased long-term efficacy. **Objectives**: An ethanolic extract of *Senna angustifolia* (EESA), a traditionally used Ayurvedic plant, was evaluated for its hair growth-promoting potential using in vivo rat models. **Methodology**: The research began with the successful extraction of phytoconstituents from *Senna angustifolia* leaves using Soxhlet extraction. The gel was formulated into two concentrations—2.5% and 5% EESA—and compared with a control and standard 2% minoxidil treatment. Hair growth studies were performed on Wistar rats by evaluating both qualitative (initiation and completion of hair growth) and quantitative parameters (hair length, follicle count, and histological observations). **Results:** Qualitative hair growth analysis indicated that both EESA concentrations accelerated hair growth, with 5% EESA (HG2) showing a significantly faster initiation and completion time (7.01 and 32.4 days, respectively) compared to control (13.8 and 42.6 days) and even slightly outperforming minoxidil (7.07 and 33.0 days). Quantitative analysis supported these findings. By day 30, HG2 achieved the greatest hair length (2.8 mm), marginally higher than minoxidil (2.4 mm) and significantly more than the control (1.2 mm). Histopathological studies corroborated these findings with increased follicle density and size. **Conclusion:** These results suggest that *Senna angustifolia* extract exhibits promising hair growth-stimulatory effects and may serve as a natural alternative or complementary treatment for alopecia with minimal irritation potential. Further clinical investigations are warranted to explore its therapeutic application in humans.

Key words: *Senna angustifolia*, Alopecia, Ayurveda, ethanolic extract, Minoxidil alternative

In mammals, hair aids visual and scent communication. Humans have less hair, mainly cosmetic. Hair loss (alopecia) affects appearance and self-esteem, ranging from patchy (alopecia areata) to complete body hair loss (alopecia universalis). Androgens cause alopecia, but treatments like finasteride and minoxidil have side effects and reduced long-term effectiveness [1-5]. Human hair is mostly fibrous α-keratin. Hair serves thermoregulation, sensing, protection, social cues, and camouflage. Humans have ~5 million follicles, 80,000–150,000 on the scalp. Hair cycles through anagen (growth), catagen (regression), telogen (rest), and exogen (shedding) stages, driven by cell differentiation, stem cell activation, and epithelial–mesenchymal interactions [6-8]. Scalp hair remains in anagen for 2–5 years, catagen for weeks, and telogen for ~3 months. Disruptions, like early catagen or shortened anagen, cause hair loss, as seen in telogen effluvium or alopecia areata due to anagen bulb damage [9]. Hair loss can have a variety of causes. The most prevalent conditions, trichotillomania, traumas, extreme stress, childbirth, usage of fertility-stimulating medications, fungal injection, hypothyroidism, sebaceous cysts, some hereditary illnesses, and short anagen syndrome are among them.

Androgenetic alopecia involves follicle shrinkage, shortened anagen, and prolonged telogen phases, while hirsutism causes excess terminal hair in females [10]. Treatment aims to modify anagen duration—shortening it for hirsutism or lengthening it for alopecia. FDA-approved drugs are topical minoxidil and oral finasteride. Minoxidil prolongs anagen and stimulates follicle growth, though its effects vary by concentration [9, 11-13]. In light of finding herbal alternatives, Senna (*Cassia angustifolia*), also called Swarna Patri in Sanskrit, an Ayurvedic herb, was employed in the current study. *Cassia angustifolia* belongs to the kingdom Plantae and the sub-kingdom Tracheobionata. It is classified under the division Magnoliophyta and the class Magnoliopsida, within the sub-

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class Rosidae. The plant falls under the **order Fabales** and is part of the **family Caesalpiniaceae**. Its **genus is Cassia**, and the **species is angustifolia**. It contains anthraquinone, a natural laxative approved by the World Health Organization (WHO) and the Food and Drug Administration (FDA). Native to Sudan but named in Arabia, Senna grows as a perennial after rains [14].

The genus Senna has 500 species; 26 contain anthracene derivatives with laxative effects. Notable ones include *Cassia angustifolia* (Alexandrine/Indian senna), *Cassia fistula*, *Cassia obovata*, and others recognized for laxative properties [14]. In the present study, we aimed to assess the quantitative and qualitative effects of *Senna angustifolia* on hair growth in rats.

MATERIALS AND METHODS

1. Identification, collection, authentication, and preparation of leaf extract

The leaves of *Senna angustifolia* were gathered in December 2019 at the Institute of Foreign Trade and Management (IFTM) University Botanical Garden in Moradabad, India. The leaves underwent air drying and washing. The Botanical Survey of India, Scientist in Charge, Allahabad (Central area), India performed the authentication. The department received a voucher specimen (Ref: BSI/CRC/Tech./2019-20/104328).

2000g powdered leaves were Soxhlet-extracted with petroleum ether and 95% ethanol for 72 hours. Extracts were filtered, concentrated at 40–50°C, and stored at 4°C.

2. Preliminary Phytochemical screening of the extract

The preliminary phytochemical screening of the extract was carried out using standard tests to identify the presence of various bioactive compounds. Flavonoids were detected using three methods: the *Shinoda Test* (formation of pink, red, green, or blue color upon addition of magnesium and hydrogen chloride), the *Alkaline Reagent Test* (yellow coloration with sodium hydroxide that turns colorless with acid), and the *Zinc-hydrogen chloride Test* (appearance of a red color). Saponins were confirmed by the *Foam Test*, where shaking the extract with water resulted in the formation of a stable 1 cm foam layer. Alkaloids were tested using *Dragendorff's reagent*, producing an orange-red precipitate, and *Mayer's reagent*, which yielded a white or cream precipitate.

For steroids and sterols, the Salkowski Test (chloroform and sulfuric acid) produced bluish-red or cherry coloration with green fluorescence, while the Libermann-Burchard Test showed a bluish-green color. Amino acids were detected by the Ninhydrin Test, resulting in a blue color upon heating. Carbohydrates were confirmed by Molisch's Test, which showed a violet ring at the interface with concentrated sulfuric acid. Proteins were identified using the Biuret Test (pink or purple color) and Millon's Test, which produced a yellow precipitate on boiling. Finally, tannins were detected using Ferric chloride (blue-black coloration) and Lead acetate, which formed a white precipitate. These tests collectively indicated the presence of multiple phytoconstituents in the extract.

3. Thin-Layer Chromatography (TLC)

TLC is a versatile analytical technique for detecting organic/inorganic compounds in micro quantities.

Plate Preparation: Silica gel G (acts as absorbent) slurry was applied to plates, dried overnight or for 30 min at 80–90 °C.

Sample Application: 1, 2, and 5 µL of MESM were spotted 2–2.5 cm from the bottom using a syringe/micropipette.

Solvent System: Non-reactive solvents were used. A 2:1:0.5 mixture of n-Hexane:Acetone was used for *Senna angustifolia* extract.

Development Chamber: A glass ascending chamber was used.

Spot Detection: Plates were placed in an iodine chamber to visualize spots.

Retardation factor (Rf) calculation:

$$Rf = \frac{\text{Distance travelled by the spots from solvent front}}{\text{Distance travelled by solvent from the origin front}}$$

4. Preparation of formulation

Three herbal gels were made using Carbopol base with methylparaben, glycerine, polyethylene glycol, polyvinylpyrrolidone, and triethanolamine. Carbopol 934 and extracts were dissolved in water, stirred (800 rpm, 1 hr), glycerin added, then triethanolamine dropwise until neutral. Stirring continued until a clear gel formed. *Senna angustifolia* extract amounts varied (Table 1).

Table 1: Preparation of formulations

Formulation	Hair gel (HG) 1	Hair gel (HG) 2	Control
Herbal Extract [g]	2. 5% of EESA	5% of EESA	---
Carbopol 934 [g]	2	2	2
Polyvinylpyrrolidone [mg]	5	5	5
Methyl paraben sodium [mg]	75	75	75
Glycerine [ml]	3	3	3
Polyethylene glycol [ml]	6.25	6.25	6.25
Triethanolamine [ml]	1.5	1.5	1.5

5. Hair growth study preparation

For the hair growth study, 150–250 g male or female rats were used from the Animal House, IFTM University. The experimental protocol was approved by the Institutional

Animal Ethics Committee (Approval No. 2019/837ac/MPH/03). Rats were kept in standard cages with proper lighting, ventilation, free access to water, and a regular diet. A 7-day acclimatisation period was observed before the study began.

5.1.1 Primary Dermal Irritation Study

Using the Organisation for Economic Co-operation and Development (OECD) 404 guidelines, Western rats were tested for skin irritation with EESA gel (2.5% and 5%). Fur was shaved from a 6 cm² dorsal area 24 hours prior. Half a gram of gel was applied, covered with gauze and tape. Erythema and edema were scored at 60 minutes, 24, 48, and 72 hours after patch removal. Primary Dermal Irritation Index (PDII) was calculated as the average irritation score over 12, 24, 48, and 72 hours: <0.5 = non-irritating; 0.5–2.0 = slightly irritating; 2.1–5.0 = moderately irritating; 5.0 = severely irritating.

6. Treatment for hair growth activity in vivo

Twenty-four rats were divided into four groups (n=6). Hair was removed from a 3 cm² dorsal area using electric shavers and a commercial remover. Group 1 (negative control) received plain gel; Group 2 (positive control) received 2% minoxidil; Groups 3 and 4 received 2.5% and 5% EESA gel, respectively. All treatments were applied topically once daily. The treatment was continued for 30 days, and the hair growth pattern was observed and tabulated.

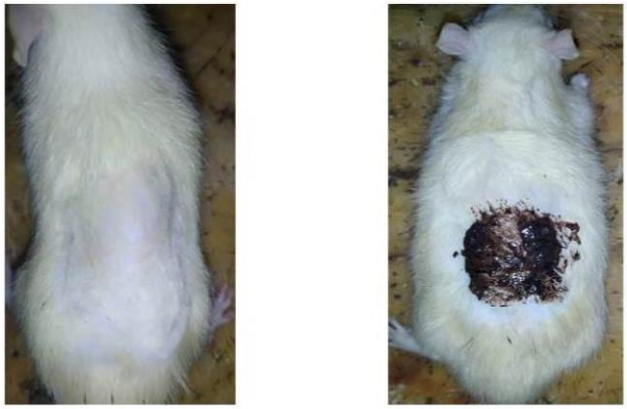

Figure 1: Treatment for Hair Growth Activity

7. Qualitative Hair Growth Study

Two parameters—hair growth initiation time (i.e., minimum time to initiate hair growth on the denuded skin region) and hair growth completion time (i.e., minimum time taken to cover the denuded skin region with new hair)—were visually observed to conduct a qualitative analysis of hair growth.

8. Quantitative Hair Growth Study

8.1 Hair length determination

Rats' shaved dorsal areas were randomly selected for hair collection on days 10, 20, and 30 of treatment using sterile forceps. After measuring the length of each hair, the findings were expressed as the mean length ± standard error of 25 hairs.

8.2 Hair follicle counting

At a constant magnification of 100x, digital photomicrographs were captured from representative sections of the slides. Following a predetermined area cropping of 1500 µm in width, each image's deep subcutis hair follicles were manually counted.

8.3 Histological observation

According to the histology observation, each treatment group will have five to seven hair follicles per millimeter of skin. However, there will be a noticeable difference between the treated and control groups in the various cycle phases (Anagen/Telogen). In all groups, around 40% of hair follicles will be in the anagen phase by the tenth day.

9. Statistical analysis

One-way ANOVA was used to analyze the data statistically for the test and control groups, and Dunnett's test was the next step. Data differences were deemed extremely significant when $P < 0.05$. GraphPad Instate version 3.06 was the computer program that was utilized. The information is presented as mean ± SEM.

RESULTS

The percentage (%) Yield of Extract from *Senna angustifolia* leaves powder was Percentage yield = 3.57 % weighing 735gm. The TLC profile is described in Table 2.

Figure 2: TLC Profile of *Senna angustifolia* extract

Table 2: TLC: *Senna angustifolia* with Rf value

Sr. No.	Solvent System	Ratio	Spot No	Colour	Rf Value
1.	n-hexane and acetone	2:0.5	1	Light yellow	0.95
2.	n-hexane and acetone	2:0.5	2	Yellow	0.88
3.	n-hexane and acetone	2:0.5	3	Light Brown	0.55
4.	n-hexane and acetone	2:0.5	4	Pale Brown	0.45
5.	n-hexane and acetone	2:0.5	5	Light Brown	0.38

Moving on to the primary dermal irritation study, using OECD scoring, no erythema or edema was observed in rats after 72 hours of herbal gel application. The PDII was zero, classifying the gel as non-irritating per OECD standards.

EESA gel was tested on telogenic rats. Anagen onset was indicated by darkening of pink skin. By day 10, HG1 (2.5%) and HG2 (5%) showed more black patches than minoxidil and the control. By day 30, full hair regrowth occurred in HG1 and HG2, while the control showed 60% regrowth. Results suggest HG2 promotes early telogen-to-anagen transition.

As for the qualitative hair growth study, over 30 days, hair growth initiation and completion were monitored with a magnifying lens. EESA 5% (HG2) treated animals showed significantly faster hair growth start and finish than the control and minoxidil groups. Control animals began hair growth at 13.8 days (Table 3).

Table 3: Effect of EESA gel formulation on hair growth initiation, completion time, and growth of hair length

Treatment	Hair Growth in days		Percent reduction in hair growth completion time	Mean length of hair in mm		
	Initiation time	Completion time	10th Day	10th Day	20th Day	30th Day
Negative Control- Simple Gel (HG3)	13.8 ± 0.42	42.6 ± 1.50	0.0 ± 0.00	0.0 ± 0.00	0.3 ± 0.0	1.2 ± 0.20
Positive Control-Standard (2% minoxidil)	7.07 ± 0.42	33.0 ± 0.71	0.6 ± 0.21	0.6 ± 0.21	1.4 ± 0.24	2.4 ± 0.20*
2.5 EESA (HG1)	11.4 ± 0.66	36.0 ± 0.45	0.1 ± 0.0	0.1 ± 0.0	1.4 ± 0.24	1.6 ± 0.24
5% EESA (HG2)	7.01 ± 0.64	32.4 ± 0.51	0.6 ± 0.20	0.6 ± 0.20	1.6 ± 0.24	2.8 ± 0.20*

*p < 0.05 significant when compared to control (ANOVA followed by Dunnett's test values are mean ± SEM

Figure 3: Hair growth on 0, 10, 20 and 30th day using HG 2

Further, hair follicles, skin thickness, and color were assessed. EESA 2.5% (HG1) showed visible changes from day 20, while EESA 5% (HG2) showed early effects by day 10. HG2 significantly enhanced hair regrowth, follicle density, and subcutis thickness, indicating telogen-to-anagen transition. H&E staining confirmed higher anagenic follicle percentages: HG2 (71%), HG1 (65.1%), minoxidil (69.4%), vs. control (47%), with significant values obtained ($p<0.05$) for HG2 and the positive control group. Increased follicle size and density confirmed HG2's superior hair growth effect.

H&E staining showed increased follicle size and number, indicating telogen-to-anagen transition. The control group had 47% anagenic follicles, while HG2 (69%) and minoxidil (69.4%) showed higher anagen rates and follicle density, confirming enhanced hair growth.

DISCUSSION

Senna (Cassia angusifolia) is commercially obtained from South India and some from the north part of India. The senna leaves contain rhein, chrysophanol, emodin, aloe emodin, mono

and diglucosides, kaemferol, palmidin, myricyl alcohol and mucilage. The leaves are commercially employed as a hair black dye [15]. The leaves have anti-dandruff, nourishing, conditioning, and strength building properties that can help to grow your hair [16].

Gasmi A et al., in 2023, described natural compounds obtained from Urtica dioica, Humulus lupulus, Serenoa repens, Vitis vinifera, Pygeum africanum, and Cucurbita pepo for reducing hair loss [17]. In another study, it was deduced that hair growth-promoting properties of phytochemicals used in various herbal compounds are associated with the tumour growth factor-β (TGF-β) pathway, β-catenin, Janus kinase-signal transducer and activator of transcription 3 J (AK/STAT3) pathway, Extracellular Signal-Regulated Kinase pathway (ERK) signaling pathway, and 5α-reductase inhibitory property. Some studies proposed that phytochemicals are more effective than conventional hair loss regimens such as minoxidil and finasteride [18].

Owing to the presence of the phytochemical properties in Senna leaf extract, ESSA was employed in the current study exploring the hair growth-promoting potential, formulated into a topical gel, and evaluated in vivo using rat models. The study demonstrated that ESSA, prepared via Soxhlet extraction (3.57% yield), contains bioactive compounds like flavonoids, tannins, glycosides, amino acids, and proteins that support hair follicle stimulation. The extract was formulated into gels (2.5% and 5%) and tested against a control and 2% minoxidil. The 5% EESA gel showed superior hair growth activity, initiating and completing growth faster than both control and minoxidil, and producing the longest hair length (2.8 mm vs. 2.4 mm with minoxidil and 1.2 mm in control). Histological analysis confirmed its efficacy, with 71% anagenic follicles, increased follicle density, and thicker skin, indicating that Senna angustifolia effectively promotes hair regeneration by stimulating the telogen-to-anagen transition.

CONCLUSION

The ethanolic extract of Senna angustifolia (EESA), when formulated as a topical gel, significantly enhanced hair growth in rats, as demonstrated by: faster initiation and completion of hair regrowth, greater hair length over time, and higher density and proportion of anagenic hair follicles, along with absence of dermal irritation or adverse effects. These results suggest that EESA—particularly at 5% concentration—is a promising, safe, and effective natural alternative for hair growth promotion. Its efficacy was found to be comparable to minoxidil, a standard FDA-approved treatment, thus supporting its potential application in the development of herbal therapies for alopecia and other hair growth disorders. Further studies, including mechanistic investigations, clinical trials in humans, and long-term safety evaluations, are recommended to validate these findings and facilitate their translation into commercial use.

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