JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/34626

RESEARCH ARTICLE

Potential Remedial Effects of *Solanum nigrum* Berries on Alopecia: An *In Vivo* Study

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Abstract

This study aimed to explore the ability of *Solanum nigrum* (*S. nigrum*) to stimulate hair growth. In this study, we investigated the effectiveness of two different extracts named methanol and petroleum ether from *S. nigrum* when applied topically. Hair loss was induced in Swiss albino rats by subcutaneously administering testosterone for 21 days. The extract was applied at the same time to assess its ability to prevent hair loss, and various measures such as follicle density, anagen/telogen (A/T) ratio, and skin section histology were monitored. Finasteride solution was used as a standard for topical application. The group treated with petroleum ether extract of *S. nigrum* showed noticeable hair regrowth, evidenced by increased (A/T) ratio, follicle density, and positive results in skin sections. The study's findings suggest that the petroleum ether extract derived from *S. nigrum* shows potential in treating hair loss induced by testosterone in experimental animals.

Keywords: Alopecia, HPLC, *In Vivo*, *Solanum nigrum*, TLC

1. Introduction

Alopecia is a dermatological condition that impacts 0.2–2% of the global population and has been well-known for more than a thousand years[1]. Androgenetic alopecia (AGA), which is influenced by androgens, affects 50% of men[2]. AGA is distinguished by the gradual transformation of normal-sized hair follicles on the scalp into smaller ones[3].

Among all the androgens, 5α-dihydrotestosterone (5α-DHT) has the greatest effect on dermal papilla cells. It is formed from testosterone within these cells by the action of 5-alpha reductase (5α-reductase or 5αR) type-2 enzyme[4]. This biocatalyst is crucial for converting testosterone to 5α-DHT within hair follicles, leading to hair loss characterized by a shortened anagen phase and shrunken hair follicles, leading to finer and shorter hairs[5].

AGA is caused by the transformation of terminal hairs toward vellus through androgen-mediated miniaturization in impaired areas of the scalp. In recent years, a variety of medical remedies and treatments have been developed, along with advances in surgical options. Currently, finasteride and minoxidil are the preferred allopathic drugs for treating AGA[3,6]. Additionally, the hair care industry is actively exploring

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Article Received on: 02.08.2023 Revised on: 11.10.2023 Accepted on: 24.11.2023

natural products, leading to a continued search for natural remedies.

Solanum nigrum, a member of the Solanaceae family, grows well in diverse soil conditions and moist environments[7]. Within the field of healthcare, *S. nigrum* is utilized for the treatment of various ailments, including tonsillitis, ringworms, pneumonia, and tumors[8,9]. This plant is widely integrated as a fundamental element in cancer treatment within traditional Chinese medicine[10]. Berries juice extract has curative properties for conditions like diarrhea, eye ailments, hydrophobia, heart disease, and anasarca. The berries are reputed to possess cathartic, tonic, and diuretic effects[11].

Sharangdhar Samhita and *Charak Samhita Grantha* mention some plants and their mixture as being historically employed in the management of *Indralupta* (Baldness) and *Khalitya* (Alopecia). The berries of *S. nigrum*, one of the botanicals employed as a remedy for alopecia[12]. To date, no scientific studies have been conducted to investigate the folklore claim that *S. nigrum* stimulates hair growth. Thus, this research was planned to examine the *in vivo* impact of *S. nigrum* extracts on hair loss triggered by testosterone.

2. Materials and Methods

2.1 Plant Material Collection and Extraction

S. nigrum Berries (SNB) were collected in February from Kolkata (West Bengal), and authenticated. Under the sun, plant materials were dried out and ground into a coarse powder. A cold maceration technique was employed to extract phytoconstituents from the pulverized barriers of *S. nigrum* using petroleum ether and methanol. The extraction was carried out at temperatures between 25 to 30°C for 3 days. The extracts were filtered using common filter paper, and the filtrates were concentrated using a rotary evaporator at temperatures between 40°C to 45°C.

2.2 Chemicals and Reagents

Sisco Research Laboratory (SRL) supplied Linoleic Acid (LA). Finasteride and Testosterone (T) were procured from Sigma-Aldrich. Merck in Mumbai supplied methanol, 95% ethanol, n-hexane, ethyl acetate, petroleum ether, and phosphoric acid. Analytical-grade chemicals were employed for all other compounds in the study.

2.3 Qualitative and Qualitative Evaluation

2.3.1 Phytochemical Investigation of Plant Extracts

By using the Singh R. and Kori ML 2022 approach, extracts were subjected to several qualitative studies to identify plant components such glycosides, saponins, proteins, carbohydrates, flavonoids, phytosterols, tannins, alkaloids, and phenolic compounds[13].

2.3.2 Identification by TLC

With a few adjustments, the TLC (thin-layer chromatography) approach from Chakraborty *et al.*, 2016, was adopted[14]. TLC was performed by using a pre-coated silica gel 60 F_{254} plate and a reference standard of LA (0.5 mg/mL dissolved in methanol). To prepare the test solution for TLC analysis, approximately 10.0 mg of the methanol extract and 3 mg of the petroleum ether extract were dissolved in the parent solvent. For standard and sample application, 4 µL of LA and 10 µL of both test samples were applied. LINOMAT V automatic sample applicator was used to spot standards and samples. As the mobile phase, a mixture of 3:2 ethyl acetate and n-hexane was utilized. The TLC plate was developed, air dried, sprayed with an anisaldehyde-sulphuric acid reagent, and heated at 105°C for 5 minutes in a hot air oven. At 540 nm, a densitometry scan was performed, and the R_f values of the standard and sample were compared.

2.3.3 HPLC Quantification

The HPLC (High-Performance Liquid Chromatography) method by Chakraborty *et al.* was used with a few modifications[15]. An Acclaim® 120 A C_{18} column from Thermo Fisher Scientific in NY, USA, measuring 4.0 mm by 250 mm by 5 µm, was used in the reversed-phase HPLC system used for separation. LA standard in methanol (0.5 mg/mL) was prepared. For calibration curve 5-20 µg/mL was prepared, for quantification of samples. Test solutions for HPLC were prepared as per the TLC method described above. The HPLC method employed an isocratic mobile phase, and the column temperature was set to 25°C. The mobile phase composition consisted of 0.5% phosphoric acid

and acetonitrile in a ratio of 90:10 (v/v). The flow rate was adjusted to 1 mL/min. After being vortexed, samples were exposed to ultrasonication for 15 minutes. The solution was then filtered through a 0.45 µ filter. A sample volume of 20 µL was injected. The detection wavelength was set at 260 nm.

2.4 *In Vivo* Study

Twenty male Swiss albino rats, aged between 6 to 8 months and weighing 130-140g, were included in the study. These rats had not received any prior drug treatments. The animals are cared for according to standard procedures, with unrestricted access to food and water. The animals were handled and cared for according to CPCSEA, India, regulations. The protocol for all animal research was approved by the institutional ethical committee of the NSHM knowledge campus, Kolkata (protocol approval no. NCPT/IAEC-010/2023).

2.4.1 Solutions

A 1% Testosterone Solution (TS) was prepared using arachis oil as the solvent. For the preparation of the extracts (2%) and the standard solution (2%) using Finasteride, a vehicle containing ethanol, propylene glycol, and water in a ratio of 8:1:1 was utilized.

2.4.2 Grouping of Animals and Treatment

Four groups of male Swiss albino rats were formed, each containing five rats (Table 1). The assignment of rats to the groups was done randomly. The treatment regimen for each group was as follows:

Table 1. Animal grouping and treatment

Group	Treatment
A	TS (Subcutaneous)+ Vehicle (Topically)
B	TS (Subcutaneous) + (2%) Finasteride solution (Topically)
C	TS (Subcutaneous) + (2%) methanol (MeOH) extract of SNB (Topically)
D	TS (Subcutaneous) + (2%) Petroleum Ether Extract (PEE) of SNB (Topically)

With very minor changes, Patel's technique (2015) was applied[3,16-18]. Rats in all groups received 0.1 mL of testosterone subcutaneously (S.C.) every day. Animals from Groups A, B, C and D received topical applications of vehicle, finasteride, and SNB extracts, respectively. Over 20 days, 0.2 mL (approximate quantity) of the vehicle or solutions was applied externally to the back skin every day. By comparing the differences in hair development in each group, the hair-growing activity of the extract was observed by visual observation. During this research, skin samples were taken at random from the bald area of each group of animals on the 7th, 14th, and 21st days following the initiation of the treatment. In a skin biopsy, follicular density, the number of hair follicles, their cyclic phase (anagen, telogen), and their anagen/telogen ratio (A/T ratio) were all measured using an ocular micrometre. For paraffin sectioning during a skin biopsy, the skin samples were preserved in phosphate-buffered formalin. Haematoxylin and eosin were used to stain vertical sections (3-4 µm) cut vertically in the direction of hair development.

3. Results

3.1 Qualitative and Qualitative Evaluation

3.1.1 Phytochemical Investigation

The MeOH extract gives a positive test of alkaloids, flavonoids, carbohydrates, protein, tannins, phytosterols, glycosides, phenolic compounds, and saponins. PEE of SNB gives a positive test of phytosterols, glycosides, protein, saponins, alkaloids and tannins.

3.1.2 TLC Identification

In TLC, linoleic acid was found in both extracts of SNB. Standard LA was shown to have an R_f value of 0.64. To verify specificity, the R_f of the standard and sample were compared (Figure 1). In the TLC plate, the concentration of LA was higher in the PEE of *S. nigrum* compared to the methanol extract spot.

3.1.3 HPLC Quantification

In HPLC, methanol extract as well as PEE of SNB were found to contain 9.73% and 32.16%, of LA. A calibration curve with the equation Y = 1015.25+ 1369x (correlation coefficient = 0.9982) was used to calculate

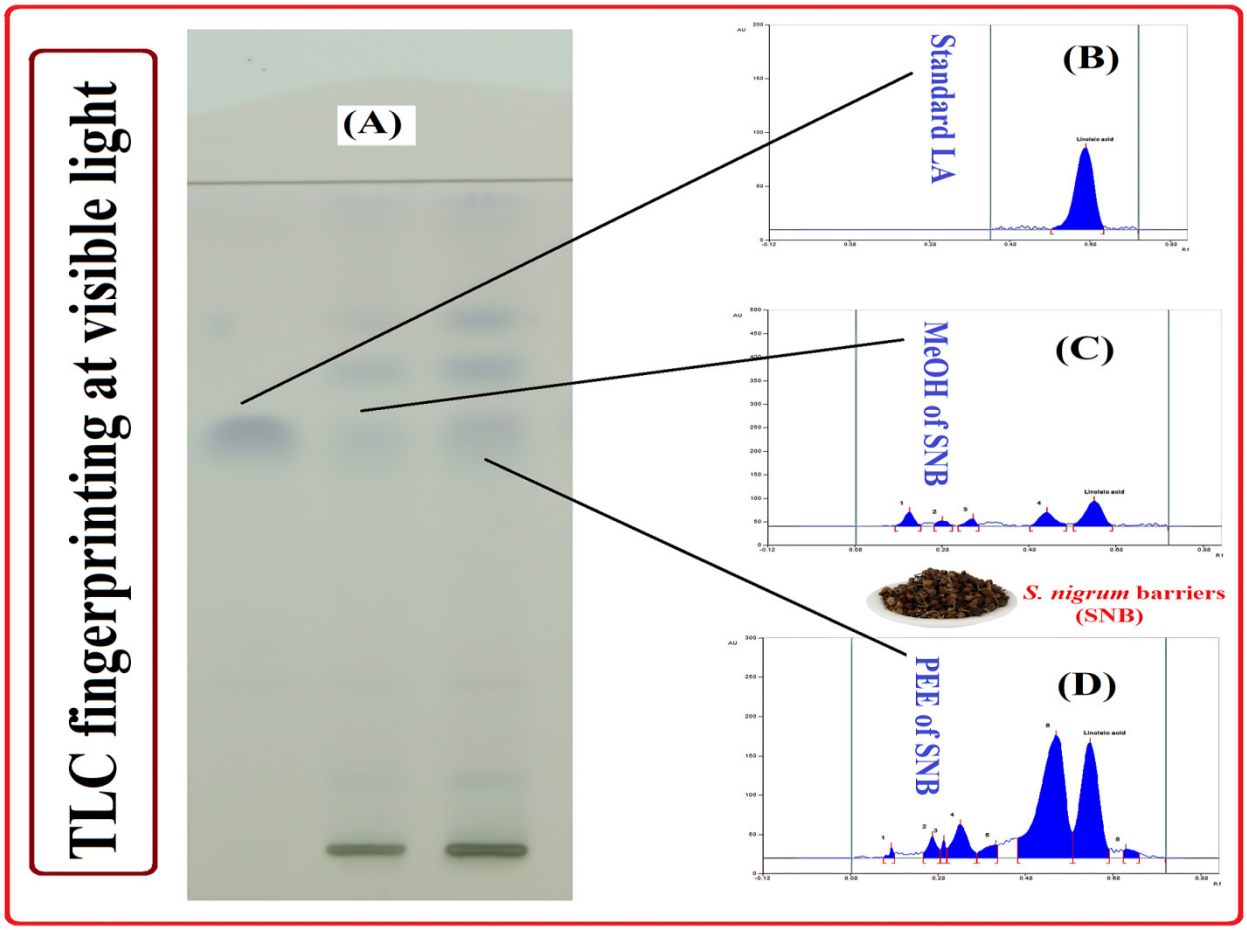

Figure 1. **(A).** TLC fingerprinting analysis at visible light, **(B).** Chromatogram of standard linoleic acid, **(C).** MeOH extract of SNB, **(D).** PEE of SNB.

the LA content shown in Figure 2A. Standard LA was shown to have an R_f value of 6.37. To verify specificity, the R_f of the standard and sample were compared (Figure 2).

3.2 Evaluation of *In Vivo* Parameters

3.2.1 Comparative Morphologic Study

Hair loss from the upper dorsum emerged on day 4 in group A rats, indicating the presence of alopecia. In contrast, Groups B, C, and D did not display any symptoms at this early stage. The control group, Group A, consistently experienced hair loss, with the region of alopecia extending to the posterior back. Animals in Groups B, C and D started losing hair from the posterior back on day 16, 10 and day 15, respectively. After the study, on day 21, Group A rats displayed diffused alopecia, whereas hair loss in the other groups remained limited to the posterior back and did not extend to the upper dorsum. Notably, these groups of animals did not exhibit any noticeable signs of alopecia, indicating that the extracts and finasteride successfully blocked the effects of testosterone and prevented testosterone-induced hair loss, as illustrated in Figure 5.

3.2.2 Tissue Histology In Vivo

3.2.2.1 Follicular Density

The microscopic images of skin samples from all the groups displayed different outcomes. The skin sections of animals treated with TS combined with the vehicle exhibited numerous hair follicles in the telogen phase. The count of telogen follicles escalated with the extension of the treatment period for the mentioned group. Skin samples from groups treated with SNB extracts and finasteride exhibited a higher number of hair follicles in the anagen phase (Figure 3). The histological findings revealed a progressive rise in

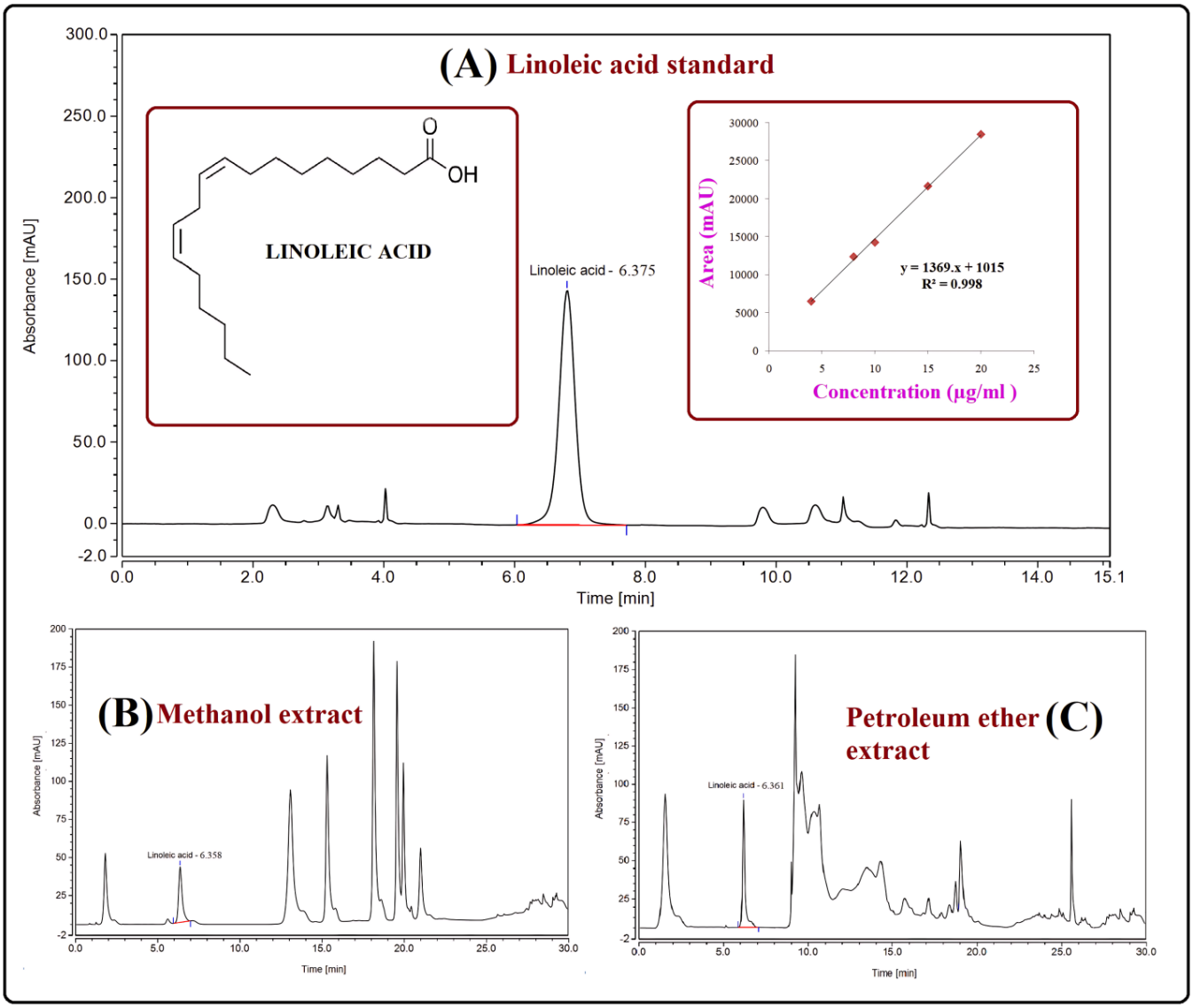

Figure 2. HPLC, **(A).** Chromatogram of standard LA; **(B).** MeOH extract of SNB; **(C).** PEE of SNB.

hair follicular density over time in the groups treated with B, C, and D. After 21 days, the finasteride-treated group exhibited the highest hair density, measuring at 3.68 ± 0.18, whereas the animals treated solely with the vehicle control group had the lowest density of 1.12 ± 0.16. The hair density in the groups treated with PEE and methanol extract of SNB was measured to be 3.18 ± 0.22 and 2.28 ± 0.16, respectively (Table 2). All data are presented as mean ± Standard Deviation (SD).

3.2.2.2 A/T ratio

The hair growth pattern in the 2% PEE extract and the finasteride-treated groups exhibited similar hair

Table 2. Follicular density of different groups at different periods of treatment

S. No.	Group	Follicular density after***		
		After 7 days	After 14 days	After 21 days
1	TS + Control Vehicle	1.42 ± 0.18	1.23 ± 0.22	1.12 ± 0.18
2	TS + Finasteride	2.68 ± 0.24	3.12 ± 0.21	3.68 ± 0.18
3	TS + SNB MeOH extract	1.73 ± 0.16	1.98 ± 0.23	2.28 ± 0.16
4	TS + SNB PEE	2.42 ± 0.26	2.96 ± 0.22	3.18 ± 0.22

Notes: Values are mean±SD, n=3. ***p<0.001, significance vs. control. TS: Testosterone solution.

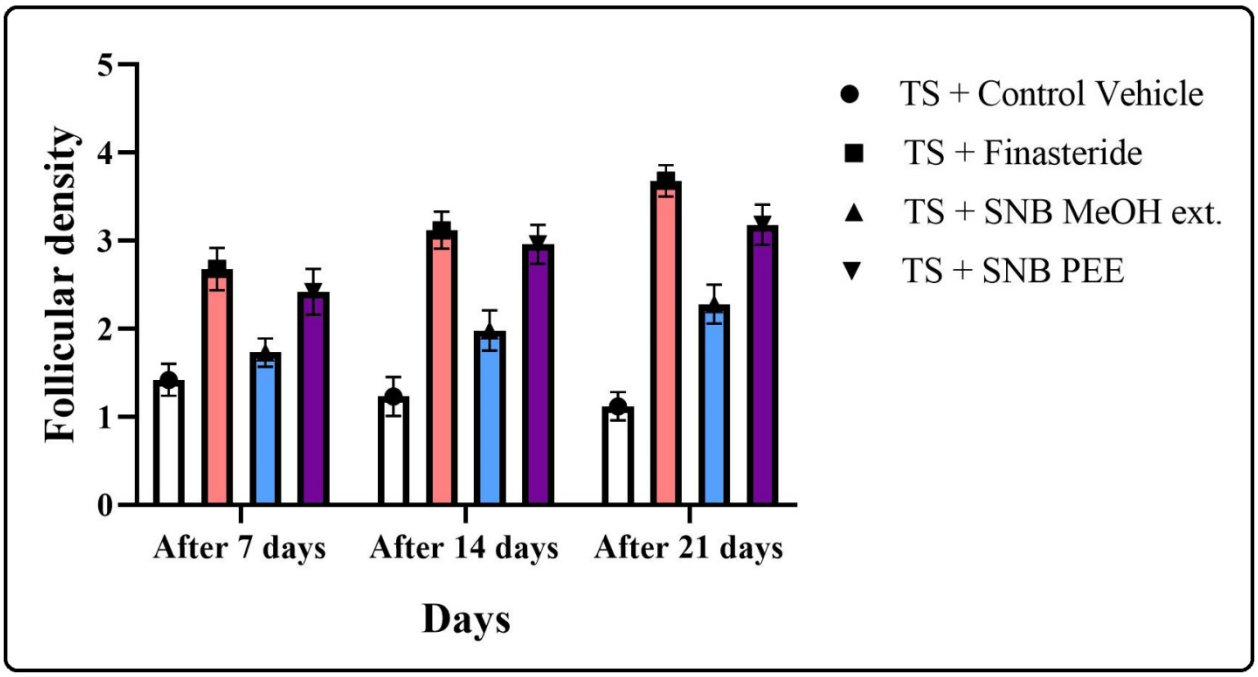

Figure 3. Follicular density in various animal groups on the 7th, 14th, and 21st days.

growth patterns. Over time, hair growth increased in both groups (Groups B, C, and D). The PEE extract was more effective in counteracting the alopecic effect of TS, rather than methanol extract. The anagen/telogen (A/T) ratio also increased in a time-dependent manner in both drug-treated groups. Notably, the A/T ratio for the 2% PEE-treated group after 21 days was 1.112 ± 0.06, while for the 2% methanol extract-treated group, was 0.726 ± 0.05. The A/T ratio for the standard drug finasteride was higher than other groups, measuring at 1.358 ± 0.04. In contrast, the A/T ratio for the control vehicle-treated group after 21 days was the lowest among all groups at 0.354 ± 0.06, attributed to the action of TS. Detailed data can be found in Figure 4 and Table 3. All data are presented as mean ± Standard Deviation (SD). Figure 5 illustrates the hair loss pattern in rat skin for different groups.

Table 3. Comparison of A/T ratio between groups

S. No.	Group	A/T ratio after***		
		After 7 days	After 14 days	After 21 days
1	TS + Control Vehicle	0.542 ± 0.06	0.477 ± 0.08	0.354 ± 0.06
2	TS + Finasteride	0.872 ± 0.05	1.029 ± 0.07	1.358 ± 0.04
3	TS + SNB MeOH extract	0.612 ± 0.04	0.702 ± 0.04	0.806 ± 0.05
4	TS + SNB PEE	0.738 ± 0.06	0.968 ± 0.06	1.112 ± 0.06

Notes: Values are mean ± SD, n=3. ***p<0.001, significance vs. control. TS: Testosterone solution.

4. Discussions

The identification of novel hair growth promoters holds immense importance due to the limited availability of FDA-approved drugs for alopecia treatment. Currently, minoxidil (topical) and finasteride (oral) are the only two options, emphasizing the need for new therapeutic interventions in this field[19].

Dihydrotestosterone (DHT) was produced when testosterone was administered to rats, resulting in AGA. DHT, known for its increased potency in comparison to testosterone, caused a decrease in the size of hair follicles, leading to the shortening of the anagen phase and a considerable extension of the telogen phase. As a consequence, thin terminal hairs were transformed into fine vellus hairs. The conversion of testosterone to DHT is primarily mediated by the enzyme 5αR type-2[20].

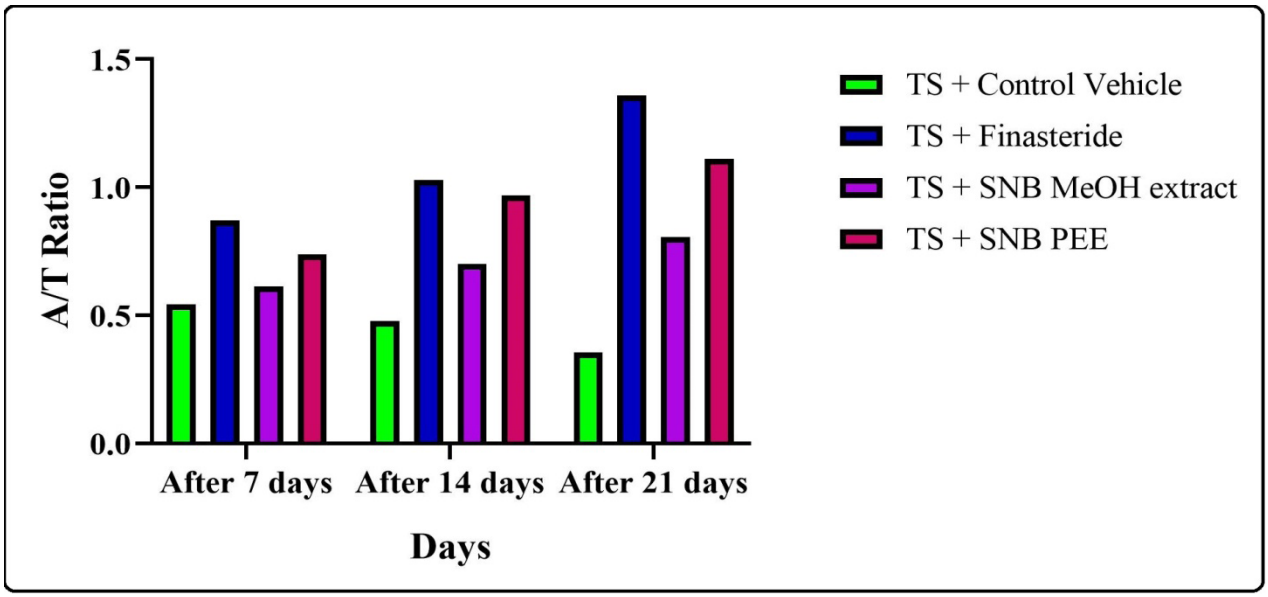

Figure 4. A/T Ratios in various animal groups on the 7th, 14th, and 21st days.

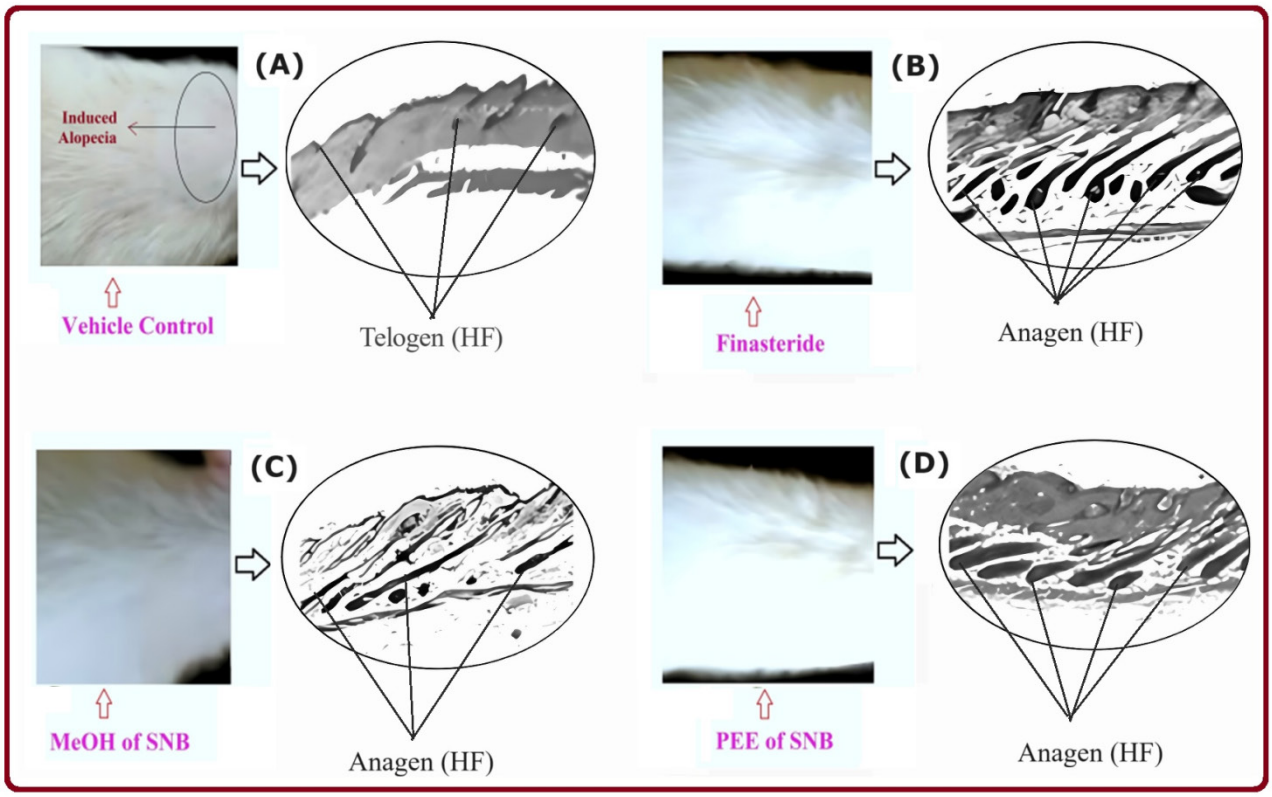

Figure 5. Hair loss pattern in rat skin by different groups after 21 days. **(A).** Group A animal, **(B).** Group B animal, **(C).** Group C animal, **(D).** Group D animal. HF: Hair follicles.

S. nigrum berries are used in the treatment of alopecia[12]. Linoleic acid is an omega-6 polyunsaturated fatty acid that has a variety of physiological properties such as 5αR, anti-anaphylactic, etc[21]. MeOH extracts as well as PEE of *S. nigrum* were found to contain 9.73% and 32.16%, of linoleic acid by HPLC. Linoleic acid is

said to be the most prevalent unsaturated fatty acid in *S. nigrum* oil[22,23].

By performing the *in vivo* study, the result suggested that *S. nigrum* has the potential to treat testosterone-induced hair loss in experimental animals by inhibiting the 5αR. The usual anagen-to-telogen ratio in healthy animals is between 6 and 8[3]. However, in cases of AGA, there is a decline in the number of anagen follicles while telogen follicles become more prevalent. This shift in follicular activity explains the increased occurrence of telogen follicles in Group A. Remarkably, the simultaneous administration of the extracts successfully reversed the testosterone-induced alopecia in rats. Among all groups, Groups B and D that received treatment with PEE of *S. nigrum* or finasteride along with testosterone, no signs of alopecia were observed. Groups B and D exhibited a higher number of anagen follicles compared to telogen follicles. This can be attributed to the inhibitory activity of the extract and finasteride on 5αR, which resulted in the prolongation of the anagenic phase and an increase in follicular density. As a result, the miniaturization of hair follicles was prevented, and the stimulation of the transformation from miniaturized, vellus-like hair to terminal hair occurred[24]. Through our study, we were able to establish the ability to stimulate hair growth of the extract by employing a combination of visual observation and quantitative data analysis in rats. Key indicators such as the hair follicular density, their cyclic phase (anagen, telogen), and A/T ratio at certain time intervals were utilized to objectively evaluate the effectiveness of the extracts.

Due to the presence of a high concentration of unsaturated fatty acids (linoleic acid, linolenic acid, and so on)[12], it may be able to associate the maximum activity of *S. nigrum*'s petroleum ether extract with its 5αR inhibitor activity. The inhibitory effect on 5αR by lipophilic extracts of *Sabal serrulata* fruits is solely attributed to the presence of free fatty acids, as proven by the demonstrated findings[25]. Isolation of linoleic acid from *Malva verticillata* seeds induced dermal papilla cell proliferation and hair development via Wnt/β-catenin signaling[26]. *Lygodium japonicum* spore (*Lygodii Spora*) extract exhibited 5αR inhibitory action and anti-androgenic effects *in vivo*. It stimulated hair growth in testosterone-treated mice, containing oleic, linoleic, and palmitic acid as primary anti-5αR components[27]. Certain unsaturated fatty acids can inhibit 5αR in cultured cells and cell-free systems. γ-linolenic acid demonstrated the highest inhibitory action, followed by arachidonic acid, α-linolenic acid, linoleic acid, palmitoleic acid, oleic acid, and myristoleic acid in decreasing order[28]. Seeds of *Sesamum indicum*, a Chinese plant used for hair development, contain significant fatty acid levels, possibly contributing to its effect on hair growth[29]. *Boehmeria nipononivea's* acetone extract inhibits 5αR and stimulates hair growth in mice. The leaf extract contains six fatty acids, including α-linolenic acid, linoleic acid, palmitic acid, elaidic acid, stearic acid, and oleic acid[30]. The primary benefit of using PEE of *S. nigrum* for alopecia lies in its ability to inhibit 5αR enzyme activity, which is attributed to its abundant linoleic acid content. The renowned 5αR inhibitory effects of free fatty acids present in saw palmetto extract, particularly linoleic acid[31], could be regarded as a potential mechanism of action for *S. nigrum*.

5. Conclusion

According to our investigation, it can be said that *S. nigrum* PEE works as an effective hair growth-promoting agent in AGA. This effect is likely attributed to its ability to inhibit the 5αR enzyme, thereby reducing the transformation of testosterone to DHT, a more active compound. It is worth noting that DHT is also implicated in the pathogenesis of other androgen-dependent conditions such as acne, prostatic cancer, and BHP. Considering the observed suppressive effect of the extract on the 5αR, it emerges as a promising candidate for managing the conditions mentioned. This significant finding not only underscores the extract's potential therapeutic benefits in treating AGA but also validates the traditional folklore claim regarding its efficacy in addressing alopecia.

6. Acknowledgements

We extend our gratitude for the invaluable assistance provided during the *in vivo* study experiment at NSHM Knowledge Campus, Department of Pharmaceutical Technology. We also express our gratitude to Ramakrishna Mission Residential College, Narendrapur, for providing us with access to the HPTLC, HPLC and other analytical instruments and other necessary facilities.

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