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EVALUATION OF HAIR GROWTH PROMOTING ACTIVITY OF *ACALYPHA INDICA* ETHANOLIC EXTRACT WHOLE PLANT

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ABSTRACT: Traditionally, alopecia, a major illness throughout the world, has been treated with medicinal plants and their oils. Hair treatments, including those that stimulate hair growth, cost about $10 billion a year. A big worry is hair loss, which is mostly brought on by junk food and erratic lifestyle choices. The function of the ethanolic extract of the entire *A. indica* plant is examined in this study. Polar components like alkaloids, flavanoids, glycosides, terpenoids, and salts were detected by qualitative screening after Soxhlet extraction. At the maximum dose of 2000 mg/kg, 14 rats in acute toxicity trials exhibited no behavioral abnormalities, a modest increase in weight, and no mortality. At 500 mg/kg and 1000 mg/kg doses, the *in-vivo* hair growth boosting activity on 24 rats demonstrated effectiveness in activating hair follicles, increasing the number of hair follicles, and promoting hair growth. The results of the study demonstrate the effectiveness of the entire *A. indica* plant and offer a formulation approach for creating hair cosmetics.

INTRODUCTION: Hair loss is a serious issue, and its frequency is concerning because of dietary and lifestyle changes, most likely stress and junk food. There are numerous documented traditional cosmetic treatments in use, including synthetic ones. However, the traditional method of using natural therapies has gained popularity, which has led to a rise in the search for natural ways to stop hair loss [1]. Alopecia is a dermatological disorder connected to hair that has been known for more than a millennium. Between 0.2 to 2% of the world's population is affected by its prevalence [2]. Nowadays, there are very few FDA-approved drugs that are used to encourage or stimulate hair growth, such as minoxidil [3].

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Minoxidil foam or topical cream has been used by both sexes with alopecia to lessen hair loss, particularly in cases of androgenic alopecia [4]. Deep insights into the traditional herbs' ability to promote hair development are provided by the tremendous efforts of scientists and writers [5]. There is currently no evidence to establish the precise mechanism of action. The majority of research indicates that the root action is the stimulation of the dermal papilla (DP) in a dose-dependent manner [6]. Mesencheymal specialized cells known as DP are found at the base of the hair follicle [7]. Through the Wnt pathway, these cells encourage progenitor root cells and stem cells to consciously go to the hair shaft and proliferate there [8].

During hair elongation, there is a significant expression of the nuclear β-catenin protein and downstream target genes of the Wnt/β-catenin pathway. The hair cycle consists of three main phases: the catagen phase, also known as the apoptotic or involution phase, which results in a marked decline in the population of epithelial cells;

the anagen or growth phase, which causes the hair shaft to elongate; and the telogen or resting phase, which completes the cycle by allowing follicle shedding and preparing the body for entry into a new anagen phase [9]. Research studies focused on hair growth promoters should include the crucial transition phases, such as anagen to catagen and telogen to anagen, since potent hair growth inducers should inhibit entry into catagen and/or stimulate transitioning to a new anagen phase [10]. Hair turns grey or white due to the same mechanism that makes it white. Our hair color gradually changes as we age. It usually affects all of the hair after beginning in the temples and progressing to the back of the head. This process affects 65% of women who are approaching forty, compared to about half of men.

Kuppameni, the Tamil name for *Acalypha indica* (Euphorbiaceae), has long been used in hair oils [11, 12]. It is still unclear what the likely underlying process for hair development is. used historically as siddha medication to heal jaundice and to rejuvenate the body. The plant's pharmacological properties, including wound healing, anticancer, antioxidant, antidiabetic, and hepatoprotective effects, are attributed to its abundance of flavanoids, glycosides, and alkaloids [8]. The current study intends to investigate the ability of the whole plant ethanolic extract of *Acalypha indica* to promote hair development.

METHODOLOGY:

Extraction and Phytochemical Investigation: Dr. Madhav Chetty of Sri Venkateswar University in Tirupati, Andhra Pradesh, recognized the entire *Acalypha indica* plant that was gathered from our institution's herbal garden and allowed to dry at room temperature. In a Soxhlet extractor, 200 grams of powdered *Acalypha indica* whole plant material was extracted using 1000 milliliters of 95% ethanol and 40 to 60 degrees Celsius [13]. Until the solvent in the thimble turns clear, the extraction will be carried on. Following an efficient extraction, a rotary evaporator (Buchi-Rota evaporator) will concentrate the solvents at room temperature under reduced pressure. Water will be eliminated by heating them over a water bath, and the extract made using each solvent will be weighed. After being stored in a desiccator, the extract was run through 40 sieves to produce a fine powder [14]. **Table 1** presents the extract's physicochemical characteristics and yield %. The powders are labeled AIE and kept in glass jars with tight-fitting lids. These extracts will be kept in the refrigerator in an amber-colored glass jar. These extracted materials will undergo a chemical analysis.

Percentage yield = weight of sample extract/ total plant material taken × 100 ------- (I)

***In-vivo* Studies:**

Drugs and Chemicals: MIJNTOP (Reddy labs) ethanolic extracts of *Acalypha indica* whole plant, distilled water, ethanol

Experimental Animals: Albino Wistar rats (150-250 g) were selected for the present study which was approved by Institutional Animal Ethics Committee (IAEC) of jeeva life sciences (JEEVA/CCSEA/IAEC/01/24) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CCSEA). Rats were kept in polypropylene cages with constant light and dark conditions, with a temperature of 22 ± 2°C and a humidity of 55 ± 5%. They were given a pellet diet and unlimited access to water. Prior to the trial, the animals were denied food for twenty-four hours, but they were given unrestricted access to water during that time [13].

Acute Toxicity: Four groups of 6 animals (Healthy Wistar albino rats either sex (180-210 g) were segregated and marked. The test extract was dissolved in distilled water and administered by oral gavage raging from 0, 500, 2000 and 5000 mg/kg dose. The behaviour and mortality of the rats was observed after dosing at initial 4hrs keenly, every 1 h during first 24 h and then for 14 days. Any change in the normal activity of rats, further their sign and symptoms of toxicity and mortality were carefully monitored and recorded. Acute toxicity study was carried out as per OECD Guideline 423 [13].

Skin Irritation Test: The mice's dorsoventral region was carefully shaved and spirit-cleaned. Additionally, a 5% ethanolic extract was applied to the mice's skin over a 2 sq cm area, and they were observed for 48 hours. Inflammation study scores were recorded, and the shaved area showed indications of primary irritation [15].

Hair Growth Promoting Activity: For the study, Swiss albino Wistar rats were chosen, and they were split into four groups of six rats each. A commercially available hair removal lotion was used to shave the rats' hairs in a 3 square centimeter region from the dorso ventral side. Surgical spirit is used to clean the shaved region. Additionally, formulations containing 100 mg/kg of extract were administered externally to the shaved area. These formulations included simple ointment and test ointment, which were made with 500 mg/kg and 1000 mg/kg of extract, respectively [16-18]. The MINItop topical minoxidil solution was bought from Reddy Laboratories, a neighborhood pharmacy in Hyderabad. The rat's dorsal surface received external applications of each solution.

- Group-I (Normal): normal control group received normal saline.
- Group-II: Test group I received AIE extract 500mg/kg.
- Group-III: Test group I received AIE 500mg/kg
- Group IV: standard Minoxidil 2% solution (MINtop).

Visual Observations: The qualitative parameters for assessing the growth of hair were defined which include hair growth initiation time (time needed to initiate hair growth), hair growth time (time taken to develop new hair).

Quantitative Parameters: The determination of hair length and diameter on the shaved area was kept in observation on 10^{th}, 20^{th} and 30^{th} day after the treatment period has started. An average of 20 hairs was collected for length and diameter. Length was measured using scale, diameter using stage micrometer. Results were expressed as Mean ± S.E.M.

Data Analysis: Statistical analysis was performed using ANNOVA and results were expressed Mean ± S.E.M. The assessment of statistical significance was done using student t test using graph pad prism 5 software. P value < 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION:

Extraction and Phytochemical Screening: The whole plant was subjected to deffating with hexane in Soxhlet apparatus and then ethanolic extraction was carried out. The percentage yield was found to be 38.9% and extract obtained was dark brown in color with semisolid texture. The phytochemical screening results have revealed the presence of alkaloids, flavanoids, glycosides, terpenoids and fatty acids.

Acute Toxicity Study: The safety dose was evaluated by the acute toxicity studies and the animals did not display any signs of toxicity, behavior changes such as rashes, inflammation, psychomotor disability and did not show any signs of mortality reported in time scale 0-3 as reported in **Table 1** at highest doses including 2000 and 5000 mg/kg. There was no much change in the body weight of the animals when the extract was administered orally. The marked body weight is represented in **Table 2** observed on 7^{th} and 14^{th} day.

TABLE 1: SIGNS OF TOXICITY

Group	Dose (mg/kg)	Signs of toxicity	Mortality
I	0	0/3	0/3
II	500	0/3	0/3
III	2000	0/3	0/3
IV	5000	0/3	0/3

0- change in behaviour 1- rashes/ inflammation 2- psychomotor disability 3 death.

TABLE 2: RESULT OF ACUTE TOXICITY- CHANGES IN BODY WEIGHT AVERAGE OF 6 RATS

Group and treatment dose	After 7 days	After 14 days
I Control	182.3±0.056	183.3±0.62
II (500mg/kg bw)	186.3±0.26	187.3±0.37
III (2000 mg/kg bw)	187.3±0.336	190.3±0.75**
IV (5000 mg/kg bw)	189.3±0.156	189.5±0.26**

The values are represented as Mean ±S. E. M (n=3) values of significance are represented as p<0.05 as * p <0/0.01 as **

Skin Irritation: No signs of primary skin irritation and inflammation were observed.

Hair Growth Promoting Activity: After the application of standard and test drug solutions the hair growth was initially observed in 14 days in control, compared to other test groups of 500 and 1000 mg/kg doses which have shown the initial growth in 7-8 days. The standard drug treatment was seen on the 7th day of the treatment as in **Table 3.** Complete hair growth was observed in the mice on the shaved area on 30th day for control and the test and standard groups have proven their efficacy by showing complete hair growth within 21 days of treatment as shown in **Fig. 2.**

The length of the hair follicle was measured and was found to be average of 20 hair follicles which was found to be 3.59. 8.56, 8.64 and 9.23 observed after the 20th, 25th and 30th days respectively for control, test samples I, II and standard. Comparable results were observed for hair length for the group treated with standard drug Minoxidil as shown in **Fig. 1**. The weight of the hairs was determined after 30 days and it was found to be 3.58 ± 0.021, 3.67± 0.056, 3.87± 0.17 and 3.86± 0.064 for test sample I, II and Minoxidil respectively as shown in **Table 4**. The normal control group was observed to have less hair weight [16].

TABLE 3: EFFECT OF *A. INDICA* ETHANOLIC EXTRACT (WHOLE PLANT) ON HAIR GROWTH- A QUANTITATIVE MEASURE OF LENGTH OF HAIR FOLLICLE

Treatment to animal groups (Topical)	After 10th Day (% Hair follicle)	After 21 days	After 28 days
Control Vehicle treated	3.10 ± 0.575	3.10 ± 0.36	3.32 ± 0.48
Minoxidil (1%)	6.53 ± 0.66****	6.66 ± 0.78 ****	6.69 ± 0.67 ****
A. indica ethanolic extract(500mg/kg)	6.83 ± 0.98 ****	6.96 ± 0.98****	6.96 ± 0.57 ****
A. indica ethanolic extract(1000mg/kg)	7.43 ± 2.5****	7.89 ± 0.65****	7.98 ± 0.98 ****

Results are represented as mean values Mean ± SEM. ∗P < 0.05, ∗∗P < 0.01, ∗∗∗P < 0.001, ns – not significant compared to control anlaysd by Student's t-test.

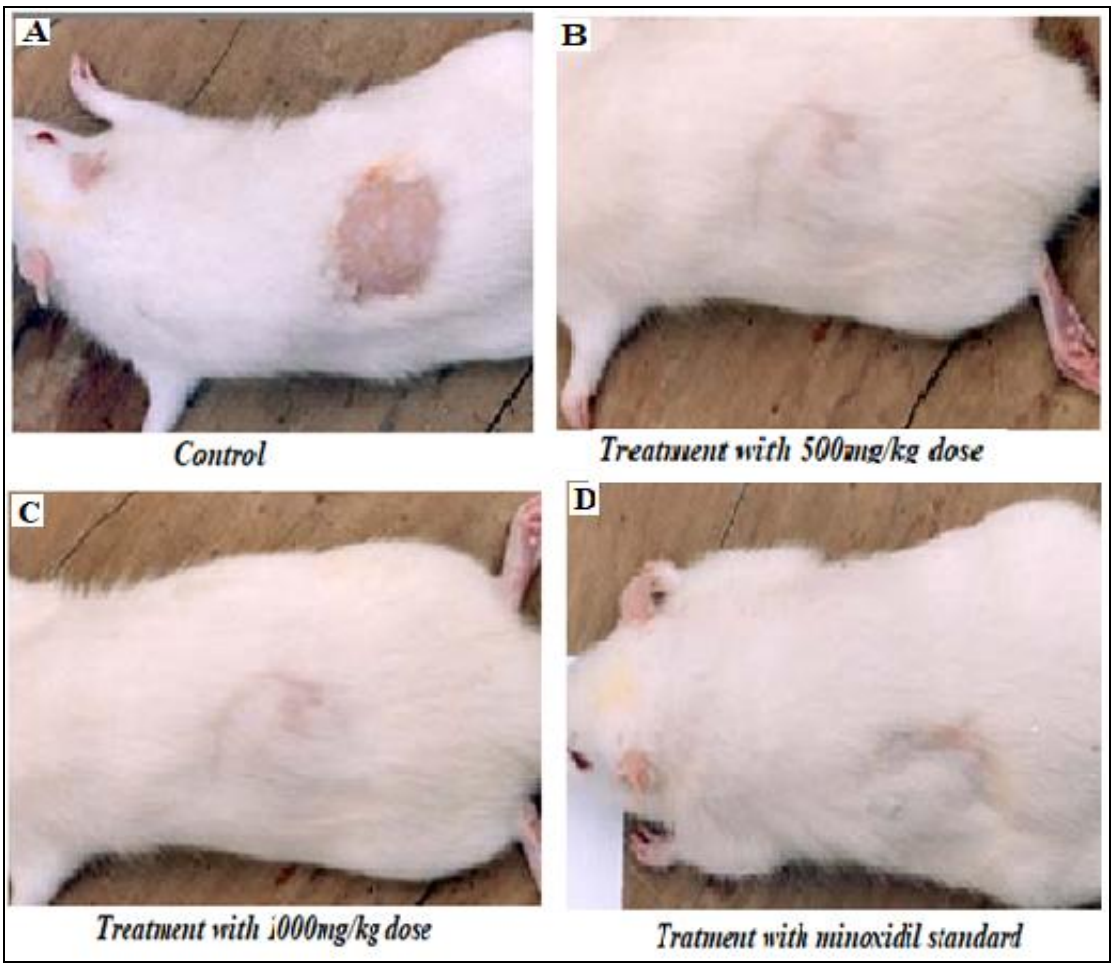

FIG. 1: EFFECT OF ETHANOLIC EXTRACT AFTER THE TREATMENT AT THE END OF 28TH DAY

TABLE 4: EFFECT OF ETHANOLIC EXTRACT OF *A. INDICA* ON INITIATION TIME AND WEIGHT OF HAIR FOLLICLE

Treatment to animal groups (Topical)	Day of initiation	Weight of hair (g)
Control Vehicle treated	10 ± 0.66	3.58 ± 0.021
Minoxidil 1 % alcoholic solution treated	8 ± 076***	3.67± 0.056
A. indica ethanolic extract (500mg/kg)	8 ± 0.84^{ns}	3.87± 0.17
A. indica ethanolic extract (1000mg/kg)	7± 0.75***	3.86± 0.064

Results are represented as mean values Mean ± SEM. ∗P < 0.05, ∗∗P < 0.01, ∗∗∗P < 0.001, ns – not significant compared to control analyzed by Student's t-test.

DISCUSSION: The plant *Acalypha indica* has no reports and supports the utility of the plant for medicinal purposes. Traditionally the plant *A. indica* has been included in the hair formulations along with other ingredients to promote hair growth and hair length. The present study was to evaluate the potential of *A. indica* whole plant ethanolic extract for its hair growth potential which was not previously reported. The ethanolic extract was obtained after defatting with hexane which completely separated the polar fraction of phytochemical *via* Soxhletion method. The qualitative screening of the extracts has revealed polar components such as alkaloids, glycosides, flavanoids, phenolics in the extract [1-3]. The ethanolic extract was investigated for acute toxicity studies based on the OECD guideline 423 [13-15]. Acute toxicity studies have proven the safety dose of the extract at highest doses i.e 2000 mg/kg and 5000mg/kg doses. The hair growth potential was explored at the doses of 500 and 1000 mg/kg doses. Significant hair growth activity was seen on the 21st and 30th day with the length of the follicles was measured using stage micrometer 33.59. 78.56, 78.64 and 89.23 µm respectively for control, test I and II followed by standard groups. Further the weight of hair follicles was measured on average of 20 and potential effect was shown by the test and standard treatments. The time for initiation of hair growth was observed on the day 7 for standard and 8th day for the tests compared to the control group observed on the 10th day [16-18]. The latent period of hair growth in high was observed for 12 days from the 10th day of initiation for both test and the standard [17]. Thus the significant contribution of the ethanolic extract of the *A. indica* at the high doses was observed in the present study. Exploration of its formulation potential in hair growth cosmetics is point of future research interest.

CONCLUSION: The study's findings support the use of the entire *Acalypha indica* plant as a hair growth promoter, and they also show that the ethanolic extract has a significant amount of potential for this purpose. The activity is attributed to the phytochemicals in the plant, including alkaloids, glycosides, flavanoids, and phenolics. The formulation aspect of using the ethanolic extract of *A. indica* has potential and scope that can help herbal cosmetics function effectively in an economical manner while also providing nontoxic benefits.

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Ethics Approval: The work was approved by Institutional Animal Ethics Committee (IAEC) of Jeeva life sciences (JEEVA/CPCSEA/IAEC/01/24)

Data Availability Statement: No data was used for the research described in the article.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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