Synergistic Trichogenic Efficacy of *Wedelia chinensis* and *Cassytha filiformis* Emulgel in Murine Models

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Abstract

Background

Herbal formulations are frequently used to treat skin diseases. Generally, a disadvantage of topical drug delivery systems is the poor permeability of the drug through the skin, due to its thick, complex structure, so large drug particles are rarely absorbed. The present study aimed to develop an *in vivo* evaluation of a herbal emulgel, alone and in combination with the plant extracts *Wedelia chinensis* and *Cassytha filiformis*, for promoting hair growth.

Methods

Hydroalcoholic extracts of *Wedelia chinensis* and *Cassytha filiformis* were used to prepare an herbal emulgel, and murine models, including testosterone- or stress-induced rats, were used to assess hair growth-promoting activity. The animals were divided into five groups of six rats each: negative control, positive control (Finasteride and Minoxidil), test group I (CF 2% emulgel), test group II (WC 2% emulgel), and test group III (CF + WC 2% emulgel).

Result

Hair growth activity was assessed visually. Biochemical and biological parameters, as well as follicular density, were measured. Histopathological analysis further revealed an increased anagen/telogen ratio in test group III (CF + WC 2%) (2.25 ± 0.6584), which was significantly higher than in the negative control group (0.25 ± 0.0156; p = ***P < 0.001). Also, it surpassed both the individual CF 2% (1.75 ± 0.5096, p = **P < 0.01) and WC 2% (1.4 ± 0.5584, p = **P < 0.01) groups. Statistical analyses were conducted in Graph Pad Prism 3 using one-way ANOVA followed by Tukey's post hoc test; *p < 0.05, ***p < 0.001 were considered significant.

Conclusion

Hydroalcoholic extract emulgel formulations for both plants exhibit significant trichogenic efficacy, but the combined formulation (CF + WC 2%) showed greater potential to promote hair growth.

Introduction

Alopecia is a distressing dermatological disorder characterised by hair loss. Although hair has no critical physiological function in humans, hair loss can be psychologically devastating and adversely affect patients' self-confidence. (Mesinkovska et al. 2023)Various factors, such as hormonal and genetic predispositions, have predominantly contributed to hair fall. (Gupta 2015)

Stress can promote the anagen-to-telogen transition and is closely associated with telogen effluvium, leading to telogen elongation. Furthermore, cortisol, the primary stress hormone, has been shown to affect the cyclic regulation of the hair cycle and proteoglycan synthesis. (Lin et al. 2020) The FDA-approved treatments for hair loss include topical Minoxidil, oral Finasteride, and low-level laser therapy. (Skrok et al. 2015) Minoxidil is converted to its active salt form (minoxidil sulphate), which activates adenosine triphosphate-sensitive potassium (KATP) channels, thereby opening them. (Bai et al. 2026) Finasteride is an inhibitor of the SRD5A2 isoenzyme, developed to treat benign prostatic hyperplasia and also used to treat AGA. 5α-Reductase inhibitors work by inhibiting 5α-reductase, which converts testosterone to dihydrotestosterone (DHT) in the presence of NADPH. This conversion enhances follicular miniaturisation and prostate enlargement, and disrupts the hair growth cycle. (Natarelli et al. 2023) (Fig. 1) From ancient times, we have seen that most products derived from plants, animals, and minerals have been used in the basic treatment of human diseases, and, given the availability of traditional medicines, people in developing countries still depend on natural products. (Erling 2016) Generally, a disadvantage of topical drug delivery systems is the poor permeability of the drug through the skin, due to its thick, complex structure, so large drug particles are rarely absorbed. However, this issue can be partially addressed by adding penetration enhancers to the emulgel formulation. Despite its benefits, emulgel is a good choice as a delivery system for hydrophobic drug substances. Emulgels are easily spreadable, easily removable, greaseless, emollient, and transparent formulations. Emulgel is a dual-release system that combines a polymer-based gel with an emulsion. This formulation is advantageous because it facilitates the delivery of both hydrophobic and hydrophilic medications into the bloodstream, thereby increasing drug release. Emulgels are prepared by incorporating an emulsion into a gel. Studies on Herbal emulgels indicate a better future for delivering a greater number of topical drugs than other drug delivery systems.(Grover & Khurana 2013)-(Dias et al. 2021)

Wedelia chinensis Merrill is a perennial herb of the family Asteraceae, commonly known as "Pilabhamgara" or "Bhringraj" in Hindi. Traditionally, the fruits, leaves, and stems are used in childbirth and to treat bites and stings, fever, and infection.(Khanum et al. 2019) The most important phytoconstituents of plants are glycosides, alkaloids, tannins, flavonoids, and phenolic compounds. The leaves are used to treat kidney dysfunction, cold, wounds, and amenorrhea. The leaves are also for dyeing hair and promoting their growth. The leaves' tonic effect is used for cough and cephalalgia. The plant's decoction is used for menorrhagia and skin diseases.(Wang et al., 2024) *Cassytha filiformis*, commonly known as Love Vine in English, belongs to the Lauraceae family. a parasitic plant distributed across many regions worldwide, including Central and South America, Africa, Asia, and the Pacific Islands, primarily in coastal areas. The plant Cassytha has over 2,500 species distributed throughout tropical and subtropical regions. This parasitic plant's main host trees include Mango, Citrus, Avocado, Nutmeg, and Clove. (Zhang et al., 2022) Many pharmacological activities of this plant have been identified so far, including diuretic, vasorelaxant, Antidiabetic, Antipyretic, Anti-inflammatory, and antitryptomania.(Sagar A et al. 2025)

This study aims to develop and assess the effectiveness of an herbal Emulgel, alone and in combination with plant hydroalcoholic extracts, and a herbal formulation containing *Wedelia chinensis* and *Cassytha*

filiformis, for promoting hair growth. *Wedelia chinensis*, a member of the Asteraceae family, was chosen for its traditional use.(Anonymous 1996) *Cassytha filiformis* from the Lauraceae family was used as a substitute for *Cuscuta reflexa* due to its significant effect on hair growth.(Sharma, S. 2009; Patel et al. 2014) Hydroalcoholic extracts of *Wedelia chinensis* and *Cassytha filiformis* contain quercetin flavonoids and phenolics, which can potentially help manage multiple diseases and their complications. The hydroalcoholic extract of *Wedelia chinensis* had the highest total flavonoid content and quercetin as compared to the hydroalcoholic extract of *Cassytha filiformis.*(Sharma et al. 2025)

Overall, the formulation in the present study was designed to assess the plant-based emulgel and evaluate the synergistic activity of extracts of *Wedelia chinensis* and *Cassytha filiformis*, which are promising for creating a skin product with trichogenic efficacy. The study aims to provide evidence on how *Wedelia chinensis* and *Cassytha filiformis* promote hair growth and to examine their potential use in treating various conditions, including testosterone and stress-induced models in rats.

Material and methods

Chemicals and Reagents

Carbopol 940 polymer obtained from Hychem Laboratory, Hyderabad.

Testosterone- Sustanon 100 mg Injection, Apollo Pharmacy

Minoxidil- Global Pharma Tek India Private Limited. Madhapur, Hyderabad

Finasteride- Hetero Drugs Limited: Bhagya Nagar Colony, Kukatpally, Hyderabad

Plant materials and preparation of the extract

The plants *Wedelia chinensis* and *Cassytha filiformis* were collected and authenticated by Professor, Department of Botany, Shri Venkateshwara University, Tirupati, India. Plants were deposited at the college as herbarium specimens. (Voucher no. 0551 & 0741) Dried aerial parts of both plants were collected and dried at room temperature in the shade. A 50 g portion of the powder was defatted with petroleum ether and subsequently macerated with hydroalcoholic (30:70) for 5 to 7 days. (Yoon et al. 2025) The plant extracts were studied for the presence of key phytochemicals implicated in the plant's action, viz., total alkaloids, flavonoids, triterpenoids, and steroids.

Preparation of hair emulgel

Separately, Carbopol 940 was dissolved in distilled water, stirred continuously to form the gel bases, and the formulation's pH was adjusted to 6-6.5 using Triethanolamine. To prepare the aqueous phase of the emulsion containing plant extract, distilled water was used to dissolve sorbitol, Tween 80,

triethanolamine, propyl paraben, and methyl paraben in propylene glycol. To prepare the oil Phase, mustard oil, paraffin liquid, and Span 80 were dissolved. The oily and aqueous phases were then separately heated to 60°C. Next, we combined the aqueous and oil phases and continuously mixed them until they reached room temperature.

Finally, the Emugel formulation (2%) was prepared by gently combining the emulsion with the gel. (Chauhan 2020) (Drapala et al. 2016) (Table 1)

Table 1
Composition of Formulation Emulgel

Ingredient	**Remarkable Nature In Formulation**	**Amount (% W/W)**		
		WC2%	**CF2%**	**CF + WC (2%)**
Carbopol 940	Gelling Agent	1	1	1
Plant Extract	(WC + CF)	2	2	2
Sorbitol	Stabilizer	0.6	0.6	0.6
Methyl Paraben	preservative	0.30	0.30	0.30
Propyl Paraben	preservative	0.01	0.01	0.01
Propylene Glycol	Co-surfactant	5	5	5
Tween 20	Non-Ionic Surfactant	0.30	0.30	0.30
Liquid Paraffin	emollient, stability	3.7	3.7	3.7
Span 80	Surfactant	1	1	1
Mustard Oil	Oil Phase	6	6	6
Triethanolamine	Ph Adjuster	q.s	q.s	q.s
Distilled Water	Volume adjuster	q.s	q.s	q.s

Stability studies

Emulgel was packed in aluminium collapsible tubes (5 g) and subjected to stability studies at 5°C/60% RH, 30°C/65% RH, and 40°C/75% RH for 3 months. Samples are withdrawn each month in accordance with ICH guidelines and analysed for physical appearance, pH, and drug release profile. (Dantas et al. 2016)

In-vivo studies on hair growth

Testosterone-Induced Model

Animals

Swiss male albino rats aged 6 to 8 months and weighing 150 to 190 gm were used in studies on testosterone-induced hair loss. All animal studies were conducted with approval from the Institutional Ethical Committee, as per the available sources.

Preparation of standard drug solution

A topical solution containing 2% minoxidil and Finasteride solution was prepared using propylene glycol, alcohol, and water (5:3:2) and applied to the animals topically for 28 days. (Li et al. 2025)

Study design

In the study, rats were divided into five groups of six animals each, with each group receiving a testosterone solution or a standard drug suspension. The following treatment was given to animals of different groups:

Group I: Testosterone solution (s.c.) + Vehicle (Topically)

Group II: Testosterone solution (s.c.) + Finasteride (Topically)

Group III: Testosterone solution (s.c.) + formulation (2%) of CF (Topically)

Group IV: Testosterone solution (s.c.) + formulation (2%) of WC (Topically)

Group V: Testosterone solution (s.c.) + formulation of (2%) CF and WC (Topically)

After an induction period of 21 days (i.e., on the 22nd day) and a treatment period of 28 days (i.e., on the 49th day), the initial and final weights were recorded, respectively. Blood was withdrawn by the retro-orbital plexus method, and biochemical parameters were measured on both the 22nd and 49th days. 3 mm^2 skin areas from two rats in each group were taken on the 22nd and 49th days. Samples were fixed in 10% formalin and sent to the laboratory for histopathological analysis. (Sarode & Gautam 2024)

Estimation of Biochemical Parameters

The cholesterol measurement enables identification of hypercholesterolemia associated with hypertriglyceridemia. The ratio should be considered when assessing one's risk of cardiovascular disease. An elevated testosterone level indicates hyperandrogenism. Blood samples collected via the retro-orbital plexus route were sent to the Pathology Laboratory for determination.

Measurement of hair length

Observe the animal to ensure that no erythema or oedema occurs within 48 hours after topical application. Hair was plucked randomly. using sterile forceps from the depilated area on the 49th day of

treatment, and the hair length was measured with the help of a scale, and the results were calculated as mean length ± SEM of 25 hairs. (Park et al. 2024)

Histological Study

On the 28th day of treatment, after receiving the standard drug and various types of test formulations, two rats from each group were selected for skin sectioning. Skin biopsies were obtained from the depilated areas of the rats and preserved in 10% formalin. (Tawanwongsri et al. 2025)

Stress-Induced Model

All albino rats of either sex were depilated with wax (2.5 cm2 area) and allowed to rest for 14 days to induce anagen. After 14 days of depilation, stress will be induced for 3 days (72 hr) using a rodent epilator (300 Hz) at 15-second intervals. After inducing stress, all albino rats were divided into 5 groups to receive their respective topical treatments, each group receiving a different formulation once daily for 7 days. Group 1 serves as the control, receiving only the vehicle, while Group 2 is treated with a 2% minoxidil drug suspension. The remaining three groups were administered experimental formulations: Group 3 received a CF2% formulation; Group 4, WC2% formulation; and Group 5, a combined CF+WC2% formulation. On the 25th day of depilation, blood samples were collected from the retro-orbital plexus to estimate biochemical parameters; skin samples (from the depilated area) for histological parameters; and hair samples for physical examination. (Ahn et al. 2023)

Hair length and Hair density determination

Hairs were plucked randomly from the depilated area of each group on the 25th day of the treatment with the help of a clipper, and the hair length of each rat was measured with the help of a vernier calliper. To assess hair density, a hole 1 to 2 centimetres in diameter was drilled in a piece of cardboard. The cardboard was placed on the desired depilated area (where hair-fall patches were observed) on the back of the rat after 25 days of depilation. The hair was trimmed of the desired depilated area, and the hair was cut with the scissors. The hairs were counted manually. (M. Kim et al. 2022)

Total Protein and Total Leucocyte Count estimation

The total protein content of homogenised tissue was measured by Lowery's method. 0.5 ml of homogenised tissue was mixed with 0.5 ml TCA 10% (trichloroacetic acid) and centrifuged for 10 min. The precipitated tissue was dissolved in 1.0 ml of 0.1 N NaOH. From this, 0.1 ml was used for the protein estimation. (Satpathy 2020) The blood was collected directly from the rat's tail. In a test tube, 0.4 mL of diluent was diluted with 20 µL of blood. The dilution is 1:20, and then the total number of cells is counted in millions per mm3 of blood. Skin biopsies were taken from the depilated area and fixed in 10% formalin. (Aksoy Sarac et al. 2023)

Statistical analysis

All data presented as MEAN ± SD of at least three samples. Statistical analysis of all data was performed using GraphPad Prism version 3.06, one-way ANOVA followed by Tukey's multiple-comparison tests.

Differences between data were considered highly significant ($P < 0.05$). The data are reported as mean ± SEM.

Results

Formulation and Evaluation of Emulgel

The emulgel was prepared by combining the emulsion and the gelling agent, with minor modifications. (Fig. 2)The prepared emulgel formulations were visually inspected for colour, homogeneity, consistency, and phase separation. (Table 2)

Table 2
Pharmaceutical Evaluation of Emulgel

Parameters	Formulations		
	WC2%	CF2%	CF + WC (2%)
Appearance	Clear	Buff	Buff
Colour	Yellowish brown	Pale Yellow	Off White
Homogeneity	Excellent	Excellent	Excellent
Extrudability	25.2	30.7	28.5
Spreadability	29.38	27.29	22.57
pH	6.8	6.5	6.2
Consistency	Excellent	Excellent	Good
Phase Separation	None	None	None
Viscosity	6812	8558	9947

Hair growth activity in the murine model (Fig. 3)

Formulations of *Wedelia chinensis* and *Cassytha filiformis* emulgels demonstrated no skin irritation in animal models after topical application. They were considered safe for topical use without causing erythema or oedema within 48 hours.

Testosterone-Induced Model

There were no significant differences in body weight on day 1 and day 22 (after the testosterone induction period), among all groups. On day 49, body weights of CF2% emulgel-treated rats were significantly lower than those of the vehicle control group (*$p < 0.05$). (Fig. 4)

Measurement of hair length

After 28 days of treatment, hair length was significantly greater in the standard treatment group and all three test formulation groups compared to the vehicle control group, as shown by statistical analysis using One-way ANOVA followed by Tukey's multiple comparisons test. (Fig. 4)

Serum Cholesterol Level and Serum Testosterone Level

After the induction period with testosterone (i.e., on Day 22), there were no significant differences in serum cholesterol levels among all groups. On day 49 (i.e. after 28 days treatment period), serum cholesterol levels were significantly decreased in the test formulation CF 2% (**P < 0.01), WC2% (**P < 0.01), CF2% + WC2% (***P < 0.001) and Standard drug (*P < 0.05) treated groups when compared to vehicle control group (Fig. 3, Table 5). After the induction period with testosterone (i.e., on Day 22), there were no significant differences in testosterone levels among groups. On day 49 (i.e. after a 28-day treatment period), serum testosterone levels were significantly decreased in all three respective test formulations, CF2%, WC2%, CF2% + WC2% (***P < 0.001) and the Standard drug (***P < 0.001) treated groups when compared to the vehicle control group. (Fig. 4)

Stress-Induced Model

Hair Length and Hair Density

Hair length was significantly increased in all three test formulation-treated groups (***P < 0.001) and the standard drug-treated group (***P < 0.001) when compared to the vehicle control group. Hair density was significantly increased in CF + WC (2%) (***P < 0.05) and standard drug-treated groups (***P < 0.001) when compared to the vehicle control group. (Fig. 5)

Total Protein and Total Leucocyte Count estimation

Total protein was significantly increased in all three test formulation-treated groups (***P < 0.001) and the standard drug-treated group (**P < 0.01) compared to the vehicle control group. The total leucocyte count was significantly decreased in all three test formulation-treated groups (***P < 0.001) and in the standard drug-treated group (***P < 0.001) compared to the vehicle control group. (Fig. 5)

Histological studies

The follicle changes in the skin were histologically analysed; the rats underwent biopsy. The group's animal skin sections, viewed under a microscope, showed that the group 1 rats were in the telogen phase, as they exhibited traits common to telogen follicles, such as being short and hollow, with more damaged follicles and shrinkage, defined as a drop in diameter rather than a deeper follicle. Compared with the control group, the herbal emulgel-treated group (CF2%, WC2%, and CF + WC 2%) and the finasteride/Minoxidil-treated group reveal reversal of the alopecia-induced changes. The herbal emulgel-treated group 5th (CF + WC 2%) showed increased follicular cell growth. (Fig. 6)

Assessment of Anagen/ Telogen ratio

The addition of the CF2% and WC2% Formulations increased dermal papilla cell proliferation compared to the negative control. Mild epidermal hyperplasia and increased thickness of the stratum basale of the epidermis of the skin. A large number of peri-follicular infiltration of inflammatory cells surrounding the follicles. Moderate proliferation of fibroblast and infiltration of immune cells [neutrophils, eosinophils and lymphocytes] in the dermal region. The (CF + WC 2%) shows moderate fibroblast proliferation; most of the follicles are in the telogen-to-anagen phase of the hair cycle. These results confirmed the hair-growth-inducing effect of the emulgel containing both plant extracts, as evidenced by the proliferation of dermal papilla cells.

Anagen telogen ratio was significantly affected by the Herbal emulgel formulation of CF2% and WC2%, which was 1.75:1 and 1.4:1, respectively, and 1.60:1 for the Finasteride/minoxidil-treated animal as against that of the testosterone–treated animal, 0.25:1. The predominance of hair follicles in the anagenic growth phase indicates the reversal of androgen-induced and stress-induced hair loss in (CF + WC 2%) treated animals, which was 2.25:1. As it is evident from the above data, the synergistic activity of *Wedelia chinensis* and *Cassytha filiformis* is comparable to that of finasteride/minoxidil. (Fig. 7)

Discussion

Numerous studies have shown that flavonoids and triterpenoids exhibit hair-growth-promoting activity by strengthening the walls of the small blood vessels supplying hair follicles, thereby improving blood circulation and nourishment, and promoting hair growth. (Bassino et al. 2020)

Hair growth results from the proliferative activity of matrix keratinocytes that form the hair shaft and inner root sheath. Matrix keratinocytes are localised in the bulb where they rest on the dermal papilla, which is a condensation of specialised mesenchymal cells with important inductive properties. The hair dermal papilla plays an important role in regulating the SHF cycle. (Lin et al. 2020). In the early stage of hair follicle growth, dermal papilla cells proliferate and differentiate to form the inner root sheath and hair shaft. When hair follicles enter a vigorous growth period, their growth rate can reach its fastest. After anagen, all hair follicles enter catagen at similar times, and their activity gradually weakens. (Liao et al. 2023) When hair follicles are ready to enter telogen from catagen, the inner root sheaths begin to undergo apoptosis. In telogen, hair follicles are considered dormant, and hair shafts stop growing and begin to shed. Some studies have shown that the telogen phase plays an important role in the hair follicle growth cycle, with its main function being to store energy and regulate and repair the skin microenvironment for hair follicle regeneration. (Skrok et al. 2015) This mechanism makes minoxidil a KATP channel opener, leading to hyperpolarisation of cell membranes, dilation of vascular smooth muscles, and increased blood circulation. The opening of KATP channels promotes HFDPC growth. Protein is crucial for reducing stress-induced hair loss. Keratin is the chief component of the hair follicle, and the hair follicle needs amino acids for protein synthesis to maintain growth. Stress increases cortisol levels, which can force hair into a resting phase; a lack of protein weakens hair follicles, leading to telogen effluvium. (Natarelli et al. 2023) Thus, cortisol inhibition may reduce telogen and promote hair growth via increased proteoglycan concentrations. (Grover & Khurana 2013)

Other researchers also implicate flavonoids in stimulating telogen to anagen phase, a process involved in hair growth and also cause expressions of some growth factors, such as insulin-like growth factor − 1 (IGF−1), vascular endothelial growth factors (VEGF), keratinocyte growth factors (KGF) and hepatocyte growth factors (HGF), all of which have stimulatory effects on hair growth. (Ashique et al. 2020) In some studies, quercetin has been reported to play a significant role in managing hair loss. Quercetin is a powerful PGD2 synthase inhibitor and thus a promising plant bioactive for development into a safe and effective pharmaceutical medication for hair loss treatment, in formulations that enable topical absorption and ensure HFs are available at the appropriate site.(J. Kim et al. 2020) By reducing DHT levels, the herbal emulgel formulation could help prevent hair follicle miniaturisation and promote hair growth. Phytoconstituents present in hydroalcoholic extracts of *Wedelia chinensis* and *Cassytha filiformis* may interact with androgen receptors or inhibit 5-alpha reductase, an enzyme that converts testosterone to DHT

Our research findings are consistent with many earlier studies on the hair-growth-promoting activity of medicinal herbs. Both plants, *Wedelia chinensis* and *Cassytha filiformis*, contain polyphenols and flavonoids. A major polyphenol has been reported to be useful in treating androgenetic alopecia by selectively inhibiting 5α-reductase activity. It could be considered that the phenols present in the extract are responsible for hair growth-promoting activity. Wedelia chinensis has the highest levels of polyphenols and quercetin among the species tested. The researchers attributed the increased hair growth to activation of the MAPK/CREB pathway, leading to increased expression of growth factors following quercetin application 48. Protein is crucial for combating stress-induced hair loss because hair follicles, primarily composed of keratin, need amino acids to maintain growth. The total protein was significantly increased in all three test formulation-treated groups, but was highest in group 5 animals treated with the combination formulation (CF + WC 2%).

For the trichogenic study, we evaluated various parameters in testosterone- and stress-induced models, including biochemical parameters (cholesterol, testosterone, leucocytes, protein) estimation; biological parameters (skin irritation, hair length, hair density) determination; histopathological studies; and estimation of the Anagen-Telogen ratio. In this study, visual observation, follicular density, and the anagen: telogen ratio were assessed to evaluate the effect of the hydroalcoholic extract on testosterone- and stress-induced hair loss. The results demonstrated that finasteride/Minoxidil and the emulgel formulation of the plants exhibit a significant effect on hair follicles. In this study, the dorsal area of albino rats treated with herbal hair emulgels showed that the CF 2% and WC 2% formulations induced the anagen phase in hair follicles in the telogen (resting) phase. Hair growth was denser in the (CF + WC 2%) treated group, which may reflect increased induction of follicles in the anagen phase. The transformation of hair follicles from the telogen phase to the anagen phase in treated groups may be due to the epithelial cell proliferation near the base of the follicles that induces vasodilatation of blood vessels in the scalp.

Histopathological analysis further revealed an increased anagen/telogen ratio in the combined group (2.25 ± 0.6584), which was significantly higher than in the negative control group (0.25 ± 0.0156; p = ***P

< 0.001). Also, it surpassed both the individual CF 2% (1.75 ± 0.5096, p = **P < 0.01) and WC 2% (1.4 ± 0.5584, p = **P < 0.01) groups. These data suggest that the combined herbal formulation produces a statistically significant enhancement in hair regrowth compared with controls and individual-extract emulgel formulations.

In summary, findings suggest that this combination, as an emulgel formulation, achieves a synergistic effect in hair regrowth; combining the two herbs (CF and WC) was more effective than using either alone. Future studies should focus on elucidating the underlying synergistic action through which *Wedelia chinensis* and *Cassytha filiformis* promote hair growth by enhancing the A/T ratio.

Conclusion

Hair growth is mediated by an undetermined cycle consisting of anagen, catagen, telogen, and exogen. A variety of factors affect the hair cycle, inducing the anagen-to-telogen transition or vice versa. Treatment with an herbal formulation had a noticeable impact on the start and completion times of hair growth in albino rats with balding skin. In this study, the hydroalcoholic extracts of *Wedelia chinensis* and *Cassytha filiformis* were formulated into an herbal emulgel and evaluated for their potential as effective topical agents to promote hair growth. The results showed that the herbal emulgel maintained appropriate pH, acceptable viscosity, and stability at room temperature. Additionally, the denuded dorsal area of albino rats treated with herbal hair emulgels prepared from these extracts showed that the combination of (CF + WC 2%) had greater potential to promote hair growth. Overall, these findings suggest that the herbal extracts could be a preferable option for future formulations.

Limitations of the study

While murine studies indicate short-term efficacy, conducting dose-dependent studies with *Wedelia chinensis* and *Cassytha filiformis* emulgel formulation could be appropriate for chronic toxicity, sensitisation, or allergic reactions resulting from long-term human use.

Abbreviations

ACTH
Adrenocorticotropic Hormone
AGA
Androgenetic Alopecia
CF
Cassytha filiformis
DHT
Dihydrotestosterone
HFDPC
Human Hair Follicle Dermal Papilla Cells

HGF
Hepatocyte Growth Factor
ICH
International Council For Harmonisation
KATP
ATP-Sensitive Potassium
KGF
Keratinocyte Growth Factor
MAPK
Mitogen-Activated Protein Kinase
NADPH
Nicotinamide Adenine Dinucleotide Phosphate
PGD2
Prostaglandin D2
SHF
Secondary Hair Follicle
TEA
Triethanolamine
VEGF
Vascular Endothelial Growth Factor
WC
Wedelia chinensis

Declarations

Ethical clearance

The study will be conducted in compliance with the requirements of the Institutional Animal Ethics Committee (IAEC). The Study plan was approved by CCSEA with protocol number No 03/IAEC-II/SLSRPL/2025, Date- 12/07/2025

Conflict of interest

The authors declare no conflict of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI.

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Figures

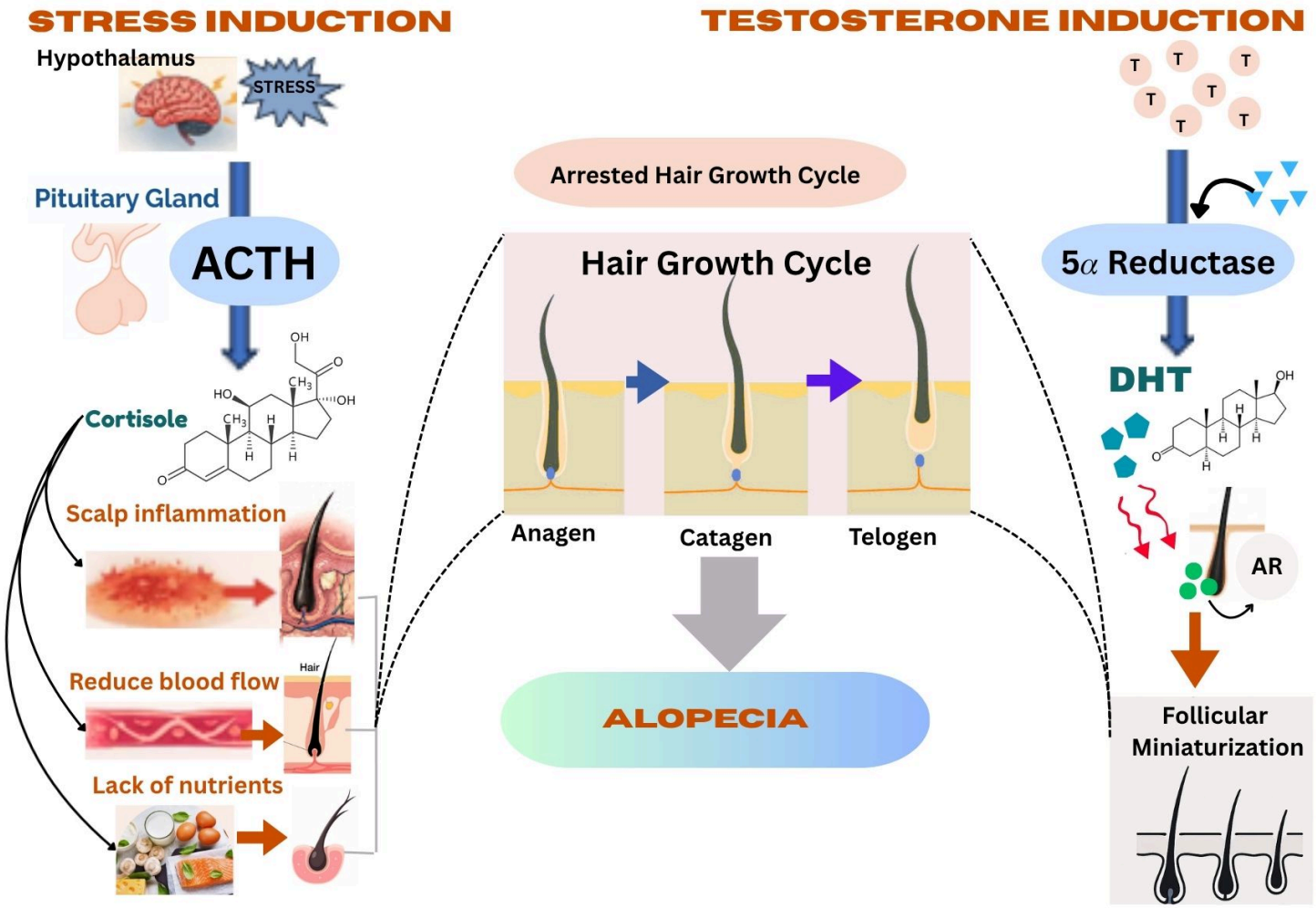

Figure 1

Mechanism of Stress-Induced and Testosterone-Induced Alopecia

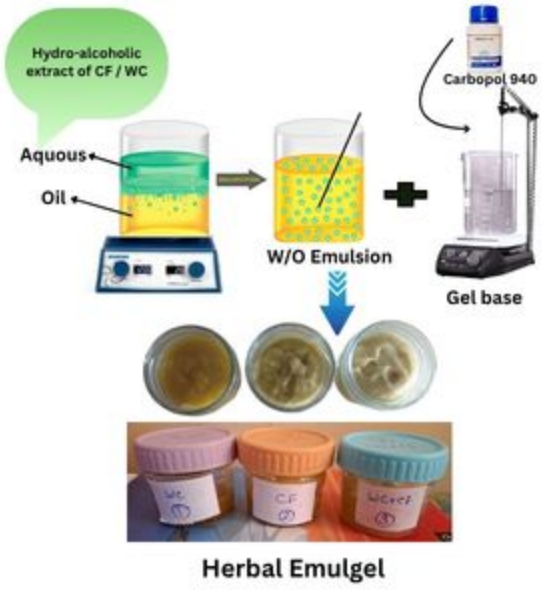

Figure 2

Herbal Emulgel Formulation

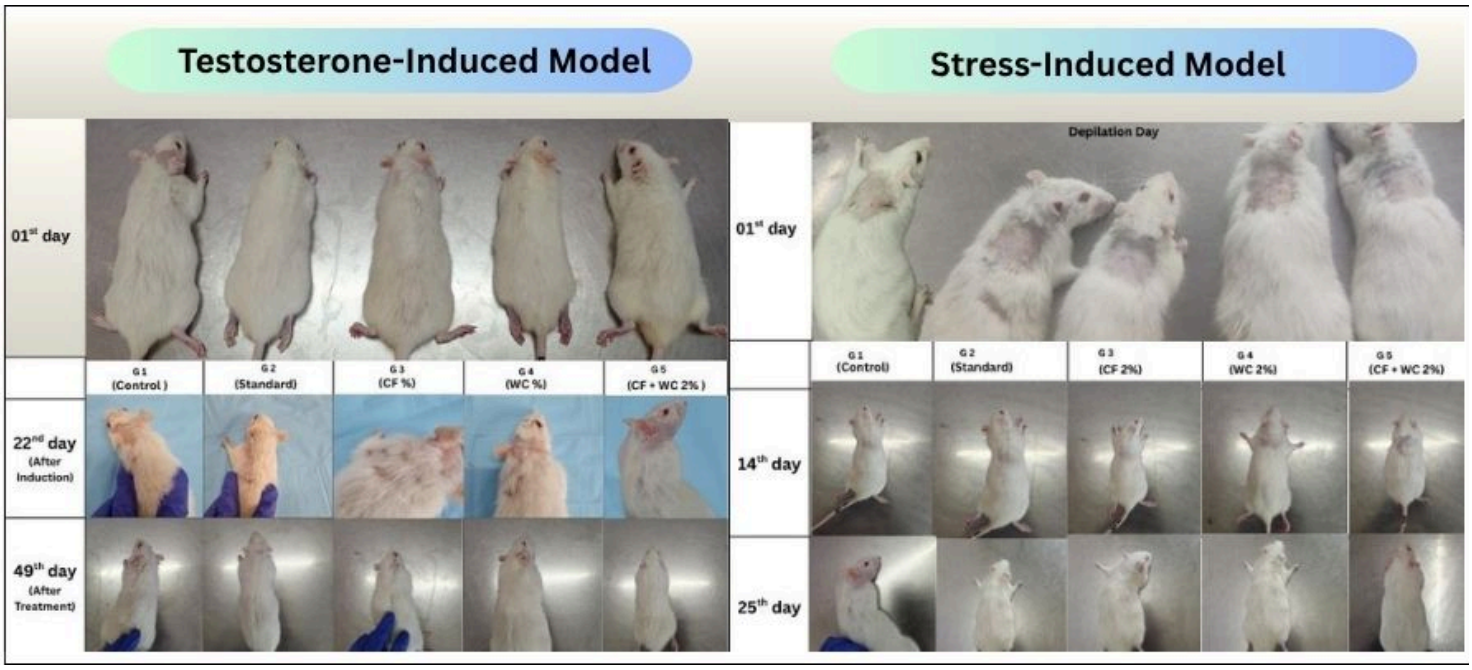

Figure 3

In vivo study for hair growth activity in the murine model

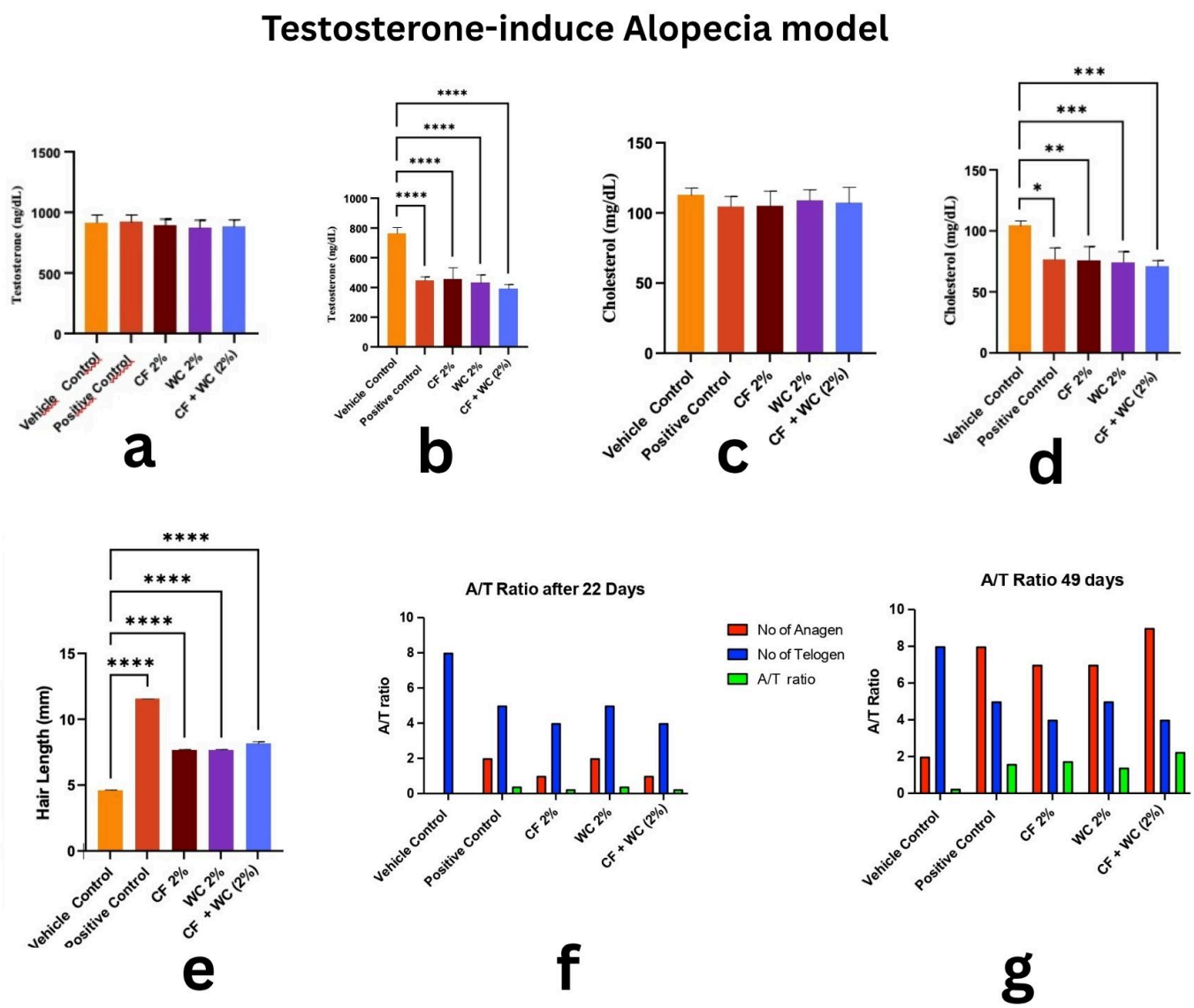

Figure 4

Testosterone induced model a) Testosterone level (mg/dL) Day 22, b) Testosterone level (mg/dL) Day 49, c) Serum cholesterol level Day 22, d) Serum cholesterol level (mg/dL) Day 49, e) A/T Ratio Day 22, f) A/T Ratio Day 49, g) Hair length determination Day 49

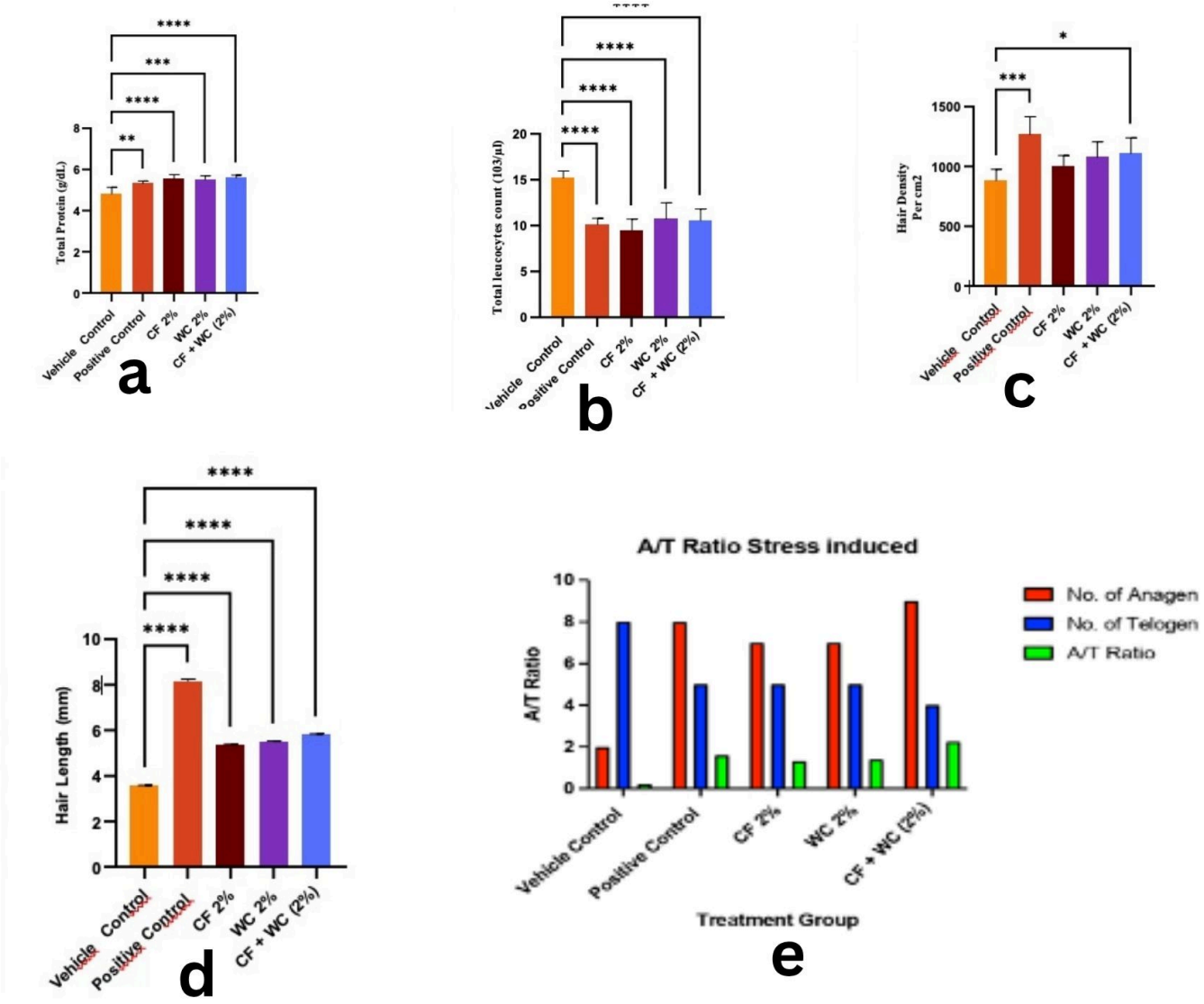

Figure 5

Stress-induced model: a) Total protein estimation, b) Total leucocyte estimation, c) Hair density determination, d) Hair length determination, e) A/T Ratio

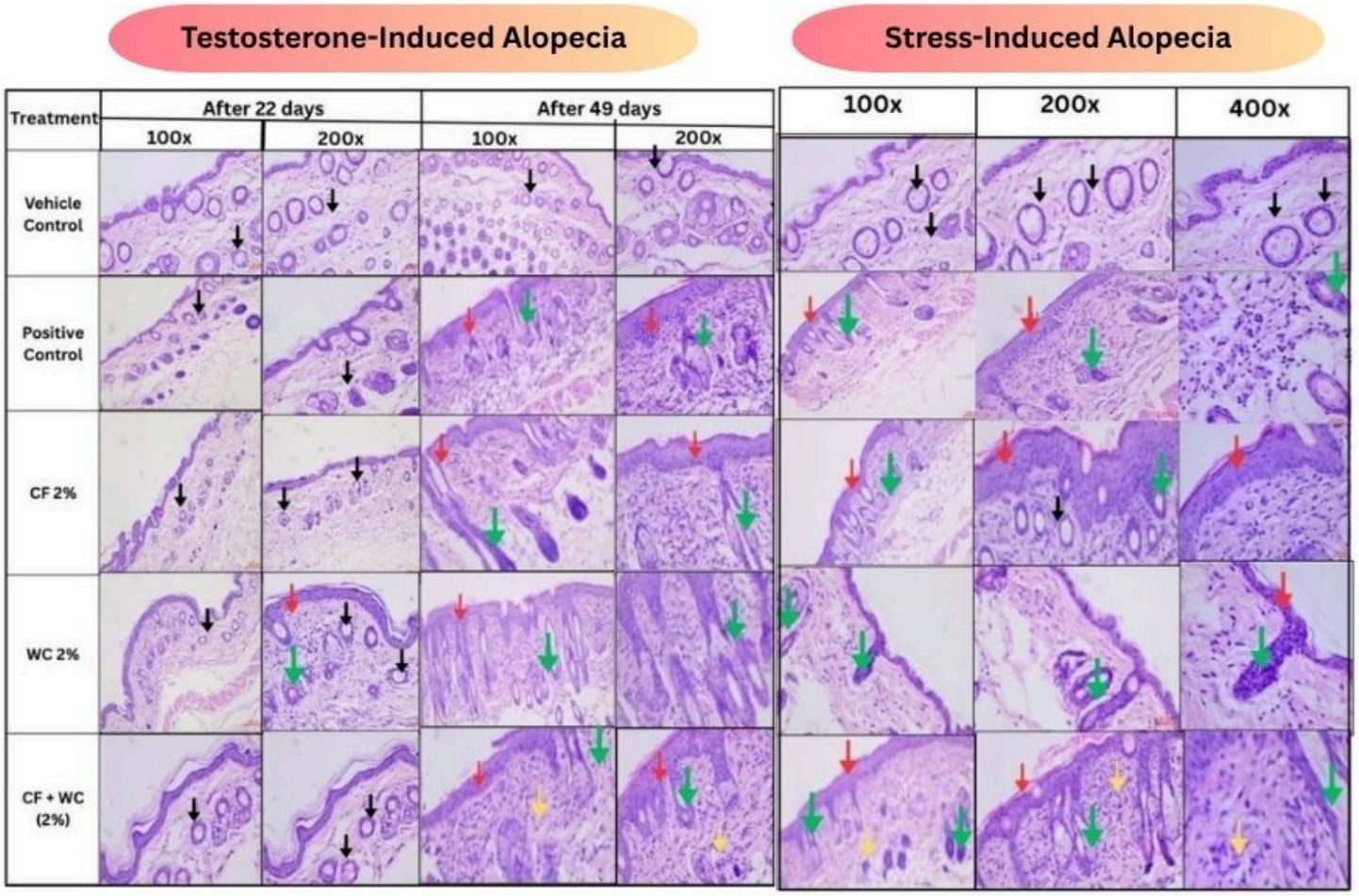
Testosterone-Induced Alopecia
Stress-Induced Alopecia
Treatment
After 22 days
After 49 days
100x
200x
100x
200x
100x
200x
400x
Vehicle Control
Positive Control
CF 2%
WC 2%
CF + WC (2%)

Figure 6

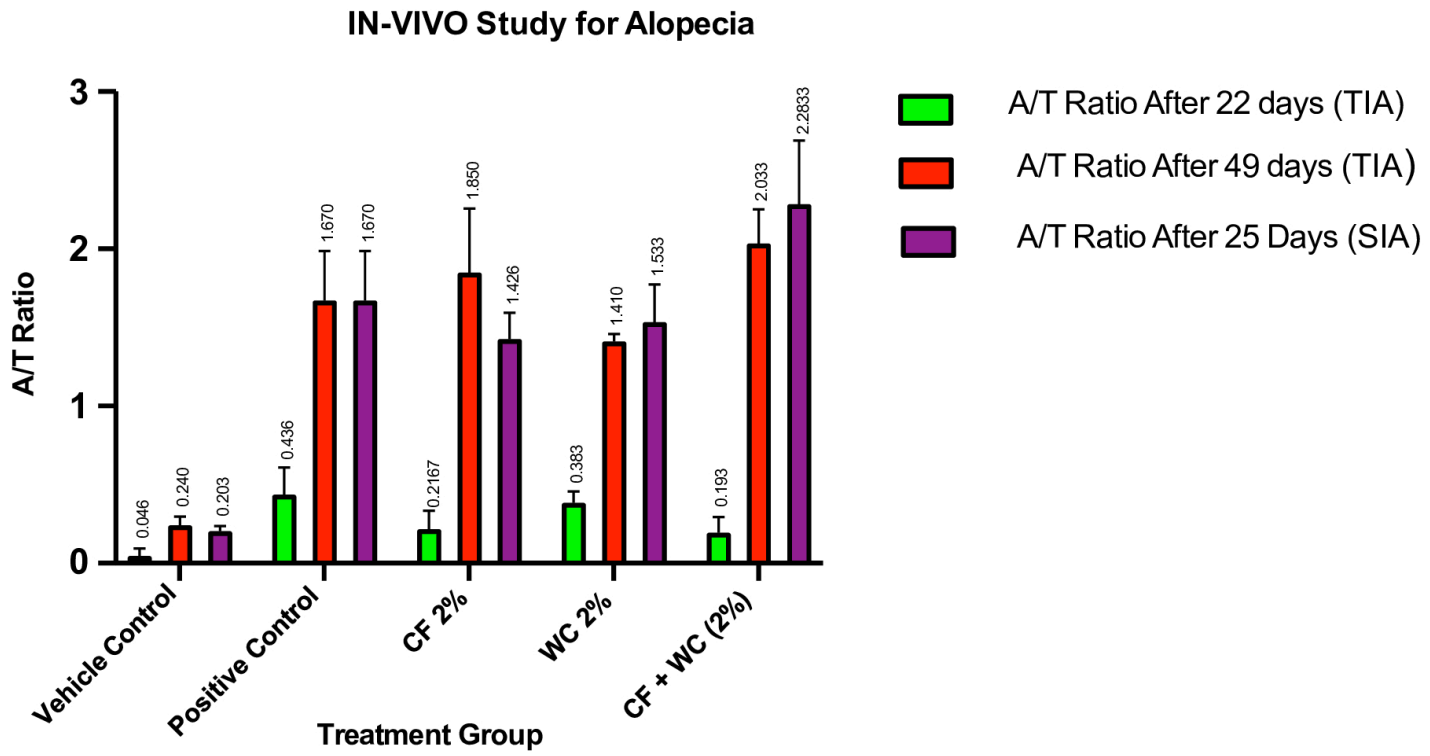

Figure 7

A/T ratio for in-vivo study of alopecia. TIA- Testosterone-induced alopecia, SIA- Stress-induced alopecia

Supplementary Files

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- Graphicalabstract.jpg