Review Article

Phytotherapeutic Interventions using Rodent Models of Hair Loss: Current Research and Future Strategy

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Hair follicles are special additions to mammalian skin with intricate differentiation and growth characteristics. They constantly undergo cycles of regression and regeneration throughout the lifespan of animal. Several rodent models have been studied for androgenetic alopecia and alopecia areata in humans with only handful of products approved by United States Food and Drug Administration; topical minoxidil (men and women) and oral finasteride (only men) for hair loss indication. This review summarizes rodent models of human hair loss to emphasize their role in testing traditional medicinal plants as hair growth promoters. Review also discusses the mechanisms of action of various herbs. Currently, both natural and synthetic products are in use to remedy alopecia. Although, use of natural products has shown measurable efficacy, the option is open to test chemically synthesized active fractions of plants as monotherapy or polytherapy to facilitate approval by regulatory agencies. Overall, purpose of this review is to discuss the rodent models of efficacy and how modifications in herbal preparations may bring more alternatives and a better value to the products in future.

Key words: Minoxidil, finasteride, rodent models, herbal preparations

Hair, a specialized appendage on body has evolved in most mammalian species as a means of protection of skin and a mode of communication via signals and cues. The outer surface of the body and limbs is covered by the epidermis, which is expanded into numerous hair follicles[1]. Hair follicles are dynamic structures owing to their growth and differentiation characteristics. After birth, mature hair follicles undergo cycles of anagen, catagen and telogen phases in a repetitive manner[2]. Major signaling molecules such as bone morphogenic protein receptor, epidermal growth factor receptor, fibroblast growth receptor 2, insulin - like growth factor 1 receptor, and fibroblast growth factor (FGF) 7 have been implicated in hair growth pattern and overall hair cycle turnover based on studies conducted using mutant mouse models of hair loss[3]. Mink skin tissues were used to demonstrate an antagonistic interaction between FGF 10 and secreted frizzled-relate protein-1 (sFRP1), both competitively regulate β-catenin pathway[4]. Such studies have shed light on genes potentially associated with similar human hair loss disorders. The quest is ongoing to understand and unravel putative mechanisms underlying different patterns of human hair loss by studying animal models[1]. Alopecia areata (AA) universalis is an autoimmune disorder that is manifested by malfunctioning of anagen phase including miniaturization of the hair follicles[5-7]. Inhibitors of Janus kinase (JAK) family protein tyrosine kinases such as ruxolitinib and tofacitinib promote rapid hair regrowth in AA in mice and humans[8]. Anagen phase dysfunction has been shown in androgenetic alopecia (AGA) (male pattern baldness) as well that is plausibly caused by the effects of dihydrotestosterone on genetically predisposed hair follicles in humans[9]. Therefore, scientific community is facing challenges to devise pharmacologically active agents that would enable hair cycle resumption already ceased in AGA. Although, minoxidil and finasteride are the only United States Food and Drug Administration (USFDA) approved products for AGA, scientists have

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raised questions on their efficacy[10]. In addition, no USFDA-approved therapy exists for AA[11].

The research to date using herbal compounds has not yielded additionally approved synthetic remedies that can combat alopecia in humans. Natural products have been proven to be source of leads for the development of drugs such as anti-cancer agents (ixabepilone and daptomycin) and anti-infectives (tigecycline and telithromycin) and, multitude of molecular biology, chemistry, and data mining approaches are aiding to create screening libraries for drug-like compounds[12]. Despite a number of clinical trials were conducted using natural products for hair loss indication, none of those products advanced to end up as approved drug products[11]. Considering researchers adopting a conventional methodology of testing racemic mixtures of different herbs in rodent models, it may be prudent to narrow down research to a level of single synthetic small molecule entity (for potential use as monotherapy or polytherapy) based on structural read outs from herbal mixtures that may enable higher probability of expanding drug pipeline for hair loss indication. Understanding mechanisms of action is one part however; establishing proof of concept in rodent model will be a critical step towards further downstream development. Generating a small molecule in a synthetic way from herbal mixture may be considered a key step to drive the drug discovery and development efforts than the natural compound getting investigated further as a potential remedy when patients are expected to receive treatment for longer duration for certain types of alopecia. The present review paper attempts to recapitulate existing preclinical research in the area of rodent efficacy models of hair loss that utilized diverse herbal preparations and emphasizes the importance of developing novel small synthetic molecules from existing natural compounds to streamline the approval process by regulatory authorities.

METHODOLOGY

The research databases such as PubMed, Scopus, Research Gate, Google, ScienceOpen, and SpringerLink were searched for the articles published from 1949 to present. Search terms included “animal models of hair loss”, “rodent models of hair loss”, ‘‘herbal products for hair loss’’, “herbal therapy for hair loss’’, and ‘‘topical herbal preparations for hair loss.” References mentioned in different reviews on herbal products and hair loss were searched for additional publications.

Animal models:

Research on animal models of human hair loss disorders started nearly seven decades ago[13]. Considering only two USFDA approved drugs for human hair loss (minoxidil and finasteride) and third repurposed drug, dutasteride for men’s hair loss approved in South Korea and Japan[14], the pursuit for novel, and efficacious hair growth promoting agents increased considerably.

Hamsters, rabbits, rodents and sheep have been used to conduct *in vivo* studies on hair loss and regeneration[13]. The *Mesocricetus auratus* (golden hamster) was used for macroscopic assessment (hair density analysis) and microscopic evaluation (hair diameter analysis) as an animal model for hair re-growth[15]. Minoxidil (5 % solution) when applied topically on frontal bald scalp of stump-tailed macaques, demonstrated hair regrowth in AGA model[16].

In general, rodent species exemplify as suitable model to study human hair loss. Mouse has short hair cycle (3 w) with well demarcated phases (anagen segmented into six and catagen into eight sub phases)[17]. There are no structural differences between mouse and human hair cycles except catagen phase in mouse shows hair bulb alterations without retraction of vibrissae follicles[1]. Rat model has ability to transform human stem cells into dermal papilla thereby culminating into formation of new hair follicles[18].

C3H/HeJ mice and Dundee Experimental Bald Rat (DEBR) models are the foundation of developing new treatments for AA[19-21]. The pigmented rodents such as C57BL/6 mice were used for studying new anagen regrowth in context of skin-free pigment and early visible pigmented tips[22]. Laser therapy, when applied for 20 s/d, 3 times per w for 6 w in C3H/HeJ mouse, induced a much longer growth phase, after only 2 w of treatment, with most of the hair follicles in anagen phase from the tested area[23]. Immunodeficient hairless outbred mice have been successfully used for development of chemotherapy induced alopecia model[24]. Keratin 5-human androgen receptor transgenic mice have been effectively tested in AGA model[25]. Patterned hair growth was studied in wild type rats and mice[26]. Human xenografts have the potential for grafting skin onto immunodeficient severe combined immunodeficiency mice[27]. *Chrysanthemum zawadskii* var. *latilobum* (Asteraceae) (CZ) stimulated hair growth in athymic nude mice by repairing nu/nu follicular keratin differentiation defect[28]. A similar efficacy was previously demonstrated in athymic nude

mice after using *Eclipta alba* extract[29]. Hair follicle bulge cells possess stem cell characteristics with a resemblance between human and mouse cells based on proliferation studies of human scalp grafted to immunodeficient mice[30].

It has been shown that topical minoxidil intervenes the normal hair cycle by shortening telogen phase, causing premature entry of the resting follicles into anagen phase thus, leads to an increased hair follicle size[31]. Finasteride on the other hand is currently marketed for AGA indication in men and acts as 5-α-reductase inhibitor[32]. Efficacy of finasteride and minoxidil are debatable[10]. Considering scarcity of approved remedies available for alopecia, an immense potential exists to expand products list. The extensive body of literature on mouse studies have already provided the evidence of genes underlying numerous human disorders, and mouse hair loss mutants are likely to benefit our understanding of human hair loss, thereby broadening our knowledge of mechanisms controlling morphogenesis[1,3] to invent better hair growth promoting agents.

Herbal Agents:

Preclinical research plays a vital role in evaluating proof of concept for any disease indication. As far as hair loss is concerned, scientists across globe have published abundant literature on herbs and their efficacious effects on rodent models of hair loss (Table 1). Rodent studies have shown beneficial effects of hair growth using herbal plants such as, 3 % proanthocyanidins from grape seeds (C3H mouse)[33], *Panax ginseng* extract and ginsenoside Rb1 (mouse vibrissal follicles in organ culture)[34], *Polygonum multiflorum* extract (C57BL/6 mice)[35], *Nardostachys jatamansi* extract and their constituents (nardal, jatamansic acid, wistar rats)[36], *Citrullus colocynthis* Schrad extract (albino mice)[37], and *Ziziphus jujuba* oil (BALB/C mice)[38]. *Eclipta alba* extract (2 and 5 %) incorporated into water in oil cream base showed a better hair growth activity than 2 % topically applied minoxidil on skin of shaved rats[39].

Herbal formulations are not always superior to standard of care and some rodent studies indicated that herbal formulation or minoxidil had similar biological activity for hair growth. Petroleum ether *Tectona grandis* seeds extract (5 and 10 %) in ointment base displayed similar increases in hair follicles in anagen phase when compared with standard of care minoxidil after 30 days of treatment in albino mice[40]. Lotion oil in water type base with 30 % of *Nicotiana tabaccum* extract[41] were equivalent in hair growth promotion as 2 % minoxidil.

Most topical herbal preparations produce stimulatory effect of hair growth promotion[42] with exception of 6-gingerol (containing main active constituent *Zingiber officinale*) that exerted proapoptotic effects on dermal papilla cells *in vitro* and caused prolongation of telogen phase *in vivo* resulting in hair growth inhibition[43].

Overall, herbal formulations have been proven to be efficacious in rodent models to promote hair growth.

Mechanisms of Action:

The molecules of interest associated with hair growth modulation are divided into two broad categories; hair growth stimulatory molecules that include Wnt/β-catenin, prostaglandin E (PGE), prostaglandin F (PGF), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and FGF-2 and FGF-7 while, hair growth inhibitory molecules include transforming growth factor beta (TGF-β), FGF-5, 5α-reductase, dihydrotestosterone (DHT), and prostaglandin D2 (PGD2)[10,44]. Herbs that have been tested thus far either showed hair growth stimulation by upregulating hair growth stimulatory molecules or they inhibited hair growth inhibitory molecules to result in hair growth promotion (Table 1). Examples of phyto constituents responsible for hair growth promoting activity are mentioned in Table 2.

FGF family are critical in human hair follicle development, epidermal differentiation and proliferation[45]. FGF-2 and FGF- 7 (identified as Keratinocyte growth factor (KGF)) positively stimulate the hair growth cycle of mice[46] while FGF-5 acts as an inhibitor of hair growth during anagen phase[47]. Lycopene isolated from rice bran supercritical CO_2 extract and other major components (linoleic acid, c-oryzanol)[48], *Lycopersicon esculentum* extracts[49], myristoleic acid from *Malva verticillata* seeds extract[50], and *Carthamus tinctorius* extract[51] are beneficial in the treatment of hair loss because of their property to stimulate KGF.

VEGF is a growth factor that stimulates vasculature and angiogenesis, thus stimulates hair growth accompanied by increases in hair follicles and hair size[52]. *Asiasari radix* extract[53], *Carthamus tinctorius* extract[51], and *Chamaecyparis obtusa* essential oils[54] induced the expression of VEGF to promote hair growth.

IGF-1 is another growth factor vital for hair growth activity. It is produced in dermal papillae and plays

TABLE 1: HERBS WITH HAIR GROWTH PROMOTING ACTIVITY IN RODENT MODELS

Plant source	Rodent species	Formulation	Mechanism of action	Reference
Aconiti ciliare	C57BL/6 mice	Aqueous Extract	Activation of Wnt/β-catenin signaling pathway	[62]
Allium tuberosum Rottler ex Spreng extract	Telogenic C57BL6/N mice	Ethanol extract	Increase the number of hair Follicles IGF-1 Upregulation	[56]
Asiasari radix	C57BL/6C3Hmice	Ethanol extract	Regulation of cell growth and growth factor gene expression VEGF upregulation	[53]
Boehmeria nipononivea	Mice	Acetone extract	Inhibition of 5α-reductase	[76]
bergamot and boxthorn	Mice	Aqueous extract	NA*	[77]
Cercidiphyllum japonicum	Mice	Methanol extract	NA*	[78]
Chamaecyparis obtusa	C57BL/6mice	Essential Oils	Positive regulator of VEGF	[54]
Crinum asiaticum	C57BL/6 mice	Ethanol extract	Proliferation of dermal papilla	[79]
Cuscuta reflexa	Swiss albino rats	Petroleum ether and ethanol extract	Promoting follicular proliferation	[80]
Eclipta alba	C57/BL6mice	Methanol extract	NA*	[81]
Eclipta alba extract	BALB/c Nude mice	Petroleum ether extract and 3 different solvent fractions in vehicle mixture	Increase in the number of follicular keratinocytes in basal epidermal and matrix cells, TGF-ßdownregulation	[29]
Erica multiflora Linn.	Mice	Ethanol extract	Promotion of dermal papilla cell growth and cell cycle	[82]
Fructus Panax ginseng	C57BL6mice	Ethanol extract	Proliferation of dermal papilla cells through anti apoptotic activation.	[35]
Ginkgo biloba	C3Hmice	Ethanol extract	NA*	[83]
Hibiscus rosasinensis	Albino rat	Petroleum ether extract	NA*	[84]
Ishige sinicola	C57BL/6 mice	Ethanol extract	5α-reductase down regulation and Wnt/β-catenin Upregulation	[61]
Lycopersicon esculentum and lycopene	C57BL/6 mice	Ethyl acetate and supercritical CO2 extracts	VEGF upregulation TGF-ß downregulation IGF-1 upregulation	[49]
Lygodii spora	C57Black/6CrSlc mice	Aqueous Ethanol extract	Inhibition of 5α- reductase	[85]
Mentha piperita	C57BL/6 mice	Peppermint oil	IGF-1 upregulation	[55]
Myricae Cortex	C57Black/6CrSlc mice	Aqueous Ethanol extract	Inhibition of 5α- Reductase	[86]
Ocimum gratissum Linn.	Sprague dawley rats C57Black/6CrSlc mice	Essential oil Aqueous Ethanol extract	Promoting follicular proliferation	[87]
Piper nigrum	C57Black/6CrSlc mice	Aqueous Ethanol extract	5α-reductase downregulation	[88]
Platycarya strobilacea S. Et Z.	C57BL/6 mice	DMSO extract	IGF-upregulation KGF- upregulation TGF-ß1 downregulation	[57]
Polygonum multiflorum	C57BL/6Nmice	Aqueous extract	Induction of ß-catenin and Sonic hedgehog (Shh)	[89]
Puerariae Flos	C57Black/6NCrSlc mice	Ethanol extract	Inhibition of 5α-reductase	[90]
Schisandra nigra	C57BL/6mice	Ethanol extract	Down regulation of TGF-ß2, proliferation of dermal papilla.	[71]
Sophora flavescent	C57BL/6mice	Methanol extract	Induction of mRNA levels of IGF-1 and KGF in dermal papilla cells, inhibition of type II 5α-reductase	[91]

Tectona grandis Linn.	Albino mice	Petroleum ether Extract	NA*	[40]
Thuja orientalis	C57BL/6N mice	Hot water extract	Wnt/ß-catenin upregulation	[92]
Zizyphus jujuba Linn.	BALB/c mice	Essential oil	NA*	[38]
Polyporus umbellatus	C3H/He mice	Methanol extract	NA*	[93]

*Not available

TABLE 2: PHYTOCONSTITUENTS RESPONSIBLE FOR HAIR GROWTH PROMOTING ACTIVITY

Biological source	Constituent	Category	References
Polyporus umbellatus Fries	3,4-dihydroxybenzaldehyde	Terpenoids	[93]
Polyporus umbellatus Fries	Acetosyringone and Polyporusterone	Steroids	[94]
Capsicum annum	Capsaicin	Alkaloid	[95]
Apple	ProcyanidinB-2	Flavonoids	[96]
Panax ginseng	Ginsenoside Ro	Saponin	[64]
Legume plants	Isoflavone	Flavones	[97]
Stephania cepharantha Hayata	Bisbenzylisoquinoline	Alkaloid	[98]
Puerariae Flos	Soya saponin I, Kaikasaponin III	Saponin	[90]
Crinum asiaticum	Norgalanthamine Nardin	Alkaloid Sesqueterpene	[79]
Nardostachys jatamansi DC	Jatamansic acid	Acid	[36]
Zingiber officinale	6-Gingerol	Flavonoid	[43]
Panax ginseng	GinsenosideF2	Saponin	[99]

an important role in the modulation of hair growth by regulating cellular proliferation and migration during hair follicle development. It has been proven that topical application of peppermint oil[55], *Allium tuberosum Rottler ex Spreng* extract[56], *Platycarya strobilacea* extract[57], and raspberry ketone from red raspberries (*Rubus idaeus*)[58] promoted IGF-1 production to positively impact hair growth activity.

EGF is expressed in the outer root sheath of hair follicles and stimulates cell propagation and formation of follicles[59]. Till to date, no preclinical studies using herbs have exclusively demonstrated promotion of EGF expression. Plants such as *Carthamus tinctorius* significantly augmented expression of hair growth promoting genes including VEGF, EGF, KGF and repressed the expression of hair growth inhibitory genes such as TGF-β1[51].

β-catenin, the transducer of Wnt signaling, is critical for the development and growth of hair follicles[60]. It was found that *Ishige sinicola* extract and its active constituent octaphlorethol A[61], *Aconiti ciliare* tuber extract[62] and *Malva verticillata* seeds extract[50] have a potential to treat alopecia via activation of β-catenin pathway.

Blockage of hair growth inhibitory activity of dihydrotestosterone (DHT) or reduction in the synthesis of DHT via inhibition of 5α-reductase activity are the principal mechanisms of herbs associated hair regrowth activity in AGA (Table 3). Both mechanisms are critical to reverse or abolish androgen dependent alopecia via inhibition of conversion of testosterone to DHT. *Thujae occidentalis* extract and linolenic acid[63], *Panax ginseng* extracts and ginsenoside Ro[64], *Cuscuta reflexa* extract[65], natural flavonoids (myricetin, quercitin, baicalein, fisetin, biochanin A, daidzein, genistein, kaempferol) and polyphenolic compounds (alizarin, anthrarobin, gossypol, nordihydroguaiaretic acid, caffeic acid phenethyl ester, octyl and dodecyl gallates)[66] have efficacious effects of inhibition of 5α-reductase activity and inhibition of conversion of testosterone to DHT in androgen dependent alopecia. *Scutellaria baicalensis* extract and its main component baicalin[67], *Rosmarinus officinalis* extract and 12-methoxycarnosic acid [68] and forsythiaside A derived from *Forsythia suspensa*[69] inhibit the binding of DHT to androgen receptors. Androgen-inducible TGF-β1 is a catagen phase inducer that mediates hair growth suppression[70]. *Eclipta alba* extract[29], *Schisandra nigra* extract[71], *Carthamus tinctorius* extract[51], and forsythiaside A derived from *Forsythia suspensa*[69] promoted hair growth via down-regulation of TGF-β expression. Treatment of HaCaT cells with the TGF-β1 inhibitors such as curcumin and apigenin resulted in a concentration-dependant decrease in TGF-β1 expression[70]. Also 1 % procyanidine B-2 from

TABLE 3: APPROVED OR UNAPPROVED (IN CLINICAL DEVELOPMENT) COMPOUNDS FOR ANDROGENETIC ALOPECIA INDICATION

Compound	Development stage	Route	Mechanism of action	Reference
Cyproterone acetate	Clinical trial	Oral	Androgen receptor antagonist	[100]
Dutasteride	Approved in Japan and South Korea	Oral, topical, mesotherapy	5α-reductase 1 and 2 downregulation	[101]
Finasteride	Approved in USA	Oral, topical, mesotherapy	5α-reductase downregulation	[102]
Fluridil	Clinical trial	Topical	Androgen receptor antagonist	[103]
Melatonin	Clinical trial	Topical	Activates prostamide a F2 receptors	[104,105]
Minoxidil	Approved in USA	Topical	Stimulates VEGF and PGE2	[106]
Spironolactone (Aldactone)	Clinical trial	Oral, topical	Reduces adrenal androgen production and competitively blocks androgen receptors	[107]
Thymosin β4	Clinical trial	Topical	Stimulates Hair Follicle stem cells	[108]
Tretinoin	Clinical trial	Topical	Promotes vascular proliferation	[109]

apple reduced TGF-β1 levels and helped in conversion of telogen follicles to anagen hair follicles[72]. PGD2 inhibition or PGE2 and PGF2 enhancement may be considered as a pharmacological mechanism for treating AGA[73]. Some active constituents from Chinese herbs such as ricinoleic acid, acteoside, amentoflavone, quercetin-3-O-rutinoside and hinokiflavone[74] and soymetide-4 derived from soy β-conglycinin[75] were predicted to be prostaglandin D2 synthase inhibitors with high efficacy and good pharmacokinetic properties for hair loss indication.

Further research on herbal products may unravel more mechanisms to target future hair growth promoting therapies.

CONCLUSION AND FUTURE RESEARCH DIRECTIONS

Hair loss is an unmet medical condition requiring therapies that will provide sustained and long-term beneficial outcome. As an example, AGA affecting adult human population is largely relying on USFDA-approved treatments such as topical minoxidil, and oral finasteride. AA is another serious disorder characterized by nonscarring hair loss secondary to immune privilege breakdown at hair follicle region with life time prevalence in population of approximately 2 %. Noteworthy is, no USFDA-approved therapy exists for AA. Complementary and alternative medicine (CAM) is one approach to invent safe, natural, and efficacious remedies to restore hair. Most often, Phytotherapeutic products are used albeit, as supplements (not requiring regulatory approval). They claim heavily about hair growth and restoration potential however, at patients level the scenario is little different. Patients seem dissatisfied with treatment outcome as there are certain demerits associated with herbal preparations including inconsistent efficacy, lack of standardized processes to determine bioactive ingredients, and paucity of product research to support efficacy claims. There has never been a dearth of diverse rodent efficacy models utilized for studying human hair loss. On most occasions, animal research proved to be beneficial with vast potential for translatability to humans. This was possible owing to our comprehensive understanding of human hair ailments, and current knowledge of approved or unapproved therapies. The caveats seem to be not associated with rodent models, instead they appear to be associated with phytotherapeutic preparations warranting modifications in existing modalities, exploring novel chemical entities or rediscovering repurposed compounds that will be maximally efficacious and minimally adverse. Furthermore, adapting a conventional approach of using racemic mixtures of herbal preparations may add more uncertainty to efficacy for certain hair loss disorders requiring an extended duration of therapy. Investing more in characterizing herbal bioactive ingredients and synthesizing single active entities to potentially serve as monotherapy or polytherapy might be the key. Synthetic small molecules can be well characterized for a physicochemical composition, purity, efficacy, minimal efficacious concentration, and toxicity profile. Same set of parameters seem difficult to get evaluated for most herbal preparations due to bigger structural heterogeneity. Therefore, active small molecule synthetic entities will have a better recognition from regulatory agencies in chemistry, manufacturing, control (CMC) and animal model efficacy areas. In light of large diversity and availability of herbal classes of compounds already tested for hair loss indication, an enormous potential exists to generate a potent and stable synthetic small molecule remedy via incessant utilization of already established or newly developed rodent efficacy models.

Conflict of interests:

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