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Research Article
Protective Effect of Ellagic Acid on Testosterone-induced Alopecia in Rats

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Abstract

Background and Objective: Alopecia or hair loss is a common dermatological problem that transcends demographic, economic, racial, gender and age barriers. There are a number of factors that affect hair growth. Amongst them, the key factors that affect hair growth are growth hormones and cytokines which are produced by the body. Ellagic Acid (EA) is a polyphenol found in a certain fruits and nuts like; grapes, pomegranate, walnuts, cranberries, raspberries, strawberries, *Morinda citrifolia* and *Terminalia chebula*. It is one of the most promising chemopreventive agents. The present study was carried out to evaluate the effect of EA on hair growth promoting activity in rats. **Materials and Methods:** The EA was studied for hair growth promoting activity in rats. Testosterone (T) administered sub-cutaneously (s.c.) for 21 days used to induce androgenetic alopecia (AGA). Minoxidil solution was applied topically served as standard. Body weight, histological parameter and hormonal parameter were estimated on post-induction day and at the end of the treatment day to observed hair growth property of EA. **Results:** The administration of testosterone leads to an increase in body weight, muscle tightness and hair loss. The EA was able to exert re-growth of hair by bringing back parameters to normal and improvement in the quantification of hair growth in rats. **Conclusion:** The EA showed a gainful outcome in testosterone-induced androgenetic alopecia in male rats. Its effects were comparable to the standard drug Minoxidil solution which is most widely used for hair growth in patients with androgenetic alopecia.

Key words: Androgenetic alopecia, hair loss, ellagic acid, minoxidil

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Alopecia is a dermatological disorder that has been recognized for more than a thousand years is a common problem in cosmetics as well as primary health care practices. It is seen all over the world and affects approximately 0.2-2% of the world population[1].

It is an emotionally distressing disease and can be caused due to different reasons, like; nutritional deficiency, aging, hormone imbalance, genetic tendencies, acute illness, expose to chemicals, medicines, chemotherapeutic agents/drugs, diabetes, auto-immune disorder, poor blood circulation, radiation exposure, skin disease, high iron deficiency, physical trauma to the scalp, other fungal infection, hair styling products, surgery and extreme stress can causes hair loss in men and women[2-5].

Androgenetic alopecia (AGA) term consists of two parts androgen (andro) and genes (genetic). It is the most common form of hair loss and often used to describe the patterned loss of scalp hair in genetically susceptible men and women[6,7].

In this condition their increase in 5α-reductase activity and 5α-dihydrotestosterone (5-α-DHT) levels. Androgens are considered to be one of the most important causes of alopecia apart from a number of genetic and environmental factors. Androgen progressively converts normal sized scalp hair follicles to miniaturized hair follicles[8].

Growth of androgen-dependent hairs can be in uenced in several ways: (a) By decreasing androgen production, (b) By blocking testosterone (T) transformation to 5-α-DHT or (c) By blocking androgen receptors[9].

Dermal papilla cells are mainly affected by 5α-DHT among all androgens. It produced from T in dermal papilla cells by the catalytic action of 5α-reductase type-2 enzymes[10].

The 5α-reductase types-2 enzymes play an important role through the intrafollicular conversion of 5α-DHT is formed by the peripheral conversion of T by 5 α-reductase[11].

In susceptible hair follicles of the scalp, 5-α-DHT binds to the androgen receptor and the hormone-receptor complex then activates the genes responsible for the gradual transformation of large, terminal follicles to miniaturized follicles[12-15].

With successive hair cycles, the duration of shortening the anagen phase and miniaturization of hair follicles become smaller, producing shorter and finer hairs that cover the scalp poorly[11]. These miniaturized hairs of various lengths and diameters are the hallmark of AGA[16,17].

Both drugs like Minoxidil (topical) and Finasterids (oral) are currently only 2 USFDA approved drugs for the treatment of AGA in men (for central/vertex hair loss only) and women (in female pattern hair loss)[18].

Now a days many Allopathic, Ayurvedic and Homeopathic product available in the market by the combination of one or more herbal drugs that find acceptability as hair tonics, hair promoting pills, hair conditioner, hair cleansing agents, hair lotions, hair oils, antidandruff agent and treatment of alopecia and or other related problems worldwide[19]. There are millions of natural products which promote hair growth i.e., fewer side-effects, easy availability, low-cost and more than one mode of action for the treatment of alopecia.

Ellagic Acid (EA) is widely distributed in woody dicotyledonous plants, where it occurs largely as ellagitannins[20-22]. The compound is known to be present in a number of soft fruits including strawberries, blackberries, raspberries[23], walnuts[24], pecans, cranberries, grapes, as well as distilled beverages. It is also found in peaches and pomegranates[25,26].

The EA plays important roles in the antioxidant activity (*In vitro* and *in vivo*). It is used to anti-proliferative[27], antimutagenic, antidiabetic[24], hepatoprotectives[28], antimicrobial[29]and antifungal[27] properties of ellagic acid have promoted research into its potential health benefits. It is used to prevent cancer and treat viral and bacterial infections.

Ellagic Acid (EA) contains 4 hydroxyl groups and lactone groups in which the hydroxyl group is known to increase antioxidant activity in lipid peroxidation and protect cells from oxidative damage. Reactive Oxygen Species (ROS) like; hydroxyl radical (OH), hydrogen peroxide (H_2O_2), superoxide anion (O^{-2}) and molecules affect both male and female gametes. The EA inhibits the generation of O_2 and OH in both enzymatic and non-enzymatic system by its metal-chelating property, thus protection against lipid peroxidation. The increase of free radicals in cells can induce the lipid peroxidation by the oxidative breakdown of polyunsaturated fatty acids in membranes of cells. The EA is a potent antioxidant acting as a scavenger of oxygen species produced by hydrogen peroxide treatment and as protector of DNA double helix from alkylating agent injury[30]. The EA is one of the natural products that reduce elevated testosterone levels, it was found that EA has promising hair growth activity. The present investigation is an attempt to show the efficacy of EA in T-induced alopecia.

MATERIALS AND METHODS

Chemicals: The EA was obtained from Muzi Agricultural Ltd., China. Minoxidil obtained from Angle Biopharma Ltd.,

Ahmedabad and all other chemicals used for experimental purpose were of analytical arcade.

Procurement of experimental animals: The experiments were carried out with Wistar albino male rats of 200-300 g bred in the animal house of the IPER, Wardha from January, 2018-December, 2019. The animals were housed in polypropylene cages at a temp. Almost 24±2°C with a relative humidity of 40-6 0% and 12 h light-dark cycle, with free access to food and water *ad libitum* during the complete study. The experimental design, animal handling and disposal procedure were approved by the institutional animal ethical committee of IPER, Wardha (IAEC/2018-19/01). All the animals were habituated for 7 days before the start of the experimental studies.

Chromatographic characterization

Chromatographic condition:

- **Principle:** High Performance Thin Layer Chromatography (HPTLC) is a suitable method for the estimation of chemical constituents present in any drugs
- **Equipment:** A Camag HPTLC system equipped with a sample applicator Linomat V, Twin trough plate development chamber, Thin Layer Chromatography (TLC) scanner III, Reprostar and WINCATS 4.02, Integration software (Switzerland)
- **Chemicals:** Analytical grade toluene, ethyl acetate, methanol and formic acid were obtained from Loba Chemicals, Mumbai

Preparation of standard EA solution: A stock solution of EA (100 µg mL^{-1}) was prepared by dissolving 10 mg of accurately weighed ellagic acid in methanol and making up the volume to 100 mL methanol. The stock solution was further diluted with methanol to give a standard solution[31] of EA (25 µg mL^{-1}).

Instrumentation and chromatographic condition: The chromatography was performed on precoated silica gel aluminum plate $60F_{254}$ (10x10 cm) with 0.2 mm thickness (E, Merk, Germany). Standard solutions of EA were prepared by transforming the stock solution in different 10 mL volumetric flask and diluted to the volume with methanol such that the concentration is 1-7 µg $µL^{-1}$. The standard and 6 different concentration of sample (EA) solution were applied on TLC plates as 6 mm width with 28 mm space between 2 bands by using a Camag Linomat V applicator. The plates were developed with a mobile phase of Toluene:Ethyl acetate:Formic acid: Methanol (3:3:0.8:0.2% v/v) in a TLC twin trough chamber previously saturated with solvent for 15 min after development the TLC plates were dried and the quantification of the standard and samples were performed by means of Camag TLC scanner III controlled by WINCATS 4.02, Integration software at 280 nm. The amount of EA in the sample solution was computed from the calibration plot (Fig. 1).

Preparation of solutions: The marketed preparation of T (Sustanone®) was diluted with arachis oil and injected to rats subcutaneously route (s.c.)[31]. The EA at concentration of 1 mg kg^{-1} dissolved in 1% CMC once daily per oral (p.o.) for 21 days and EA was diluted with propylene glycol and water were applied topically and the standard of Minoxidil (3%) was diluted with propylene glycol, alcohol and water were applied topically.

Experimental design: The present study was conducted by using male Wistar albino rats divided into 8 groups (containing 6 rats in each group). Normal (Group-I) animals received only vehicle (normal saline solution once daily, p.o). The control (Group-II) and 6 treatment groups (Group-III,

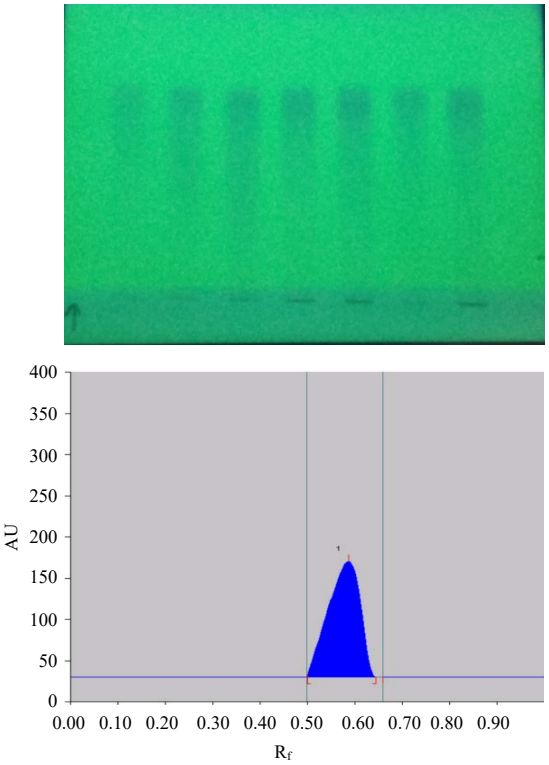

Fig. 1: HPTLC fingerprints showing peak area of ellagic acid
R_f value of ellagic acid was found to be 0.58

Group-IV, Group-V, Group-VI, Group-VII and Group-VIII) rats were injected with T which was diluted with arachis oil once daily s.c. for 21 days to induce rat model of alopecia. The control group was left for natural recovery for 21 days post T-treatment. The standard group animals were applied Minoxidil (3%) topically for 21 days. Group-IV, Group-V and Group-VI animals were administered with EA (10, 20 and 30 mg kg^{-1}) once daily (p.o.) for 21 days. Group-VII animals received EA (topical) and Group-III animals received Minoxidil 3% daily topical for 21 days. Group-VIII animal received Minoxidil 3% and EA (10 mg kg^{-1}) in combination once daily for 21 days.

Approximately 0.1-0.2 mL of solution topically applied on back skin once a day for 21 days. On 22nd day, rat from each group selected randomly and sacrificed. The difference in the growth of hair in each group noticed by visual observation and recorded by taking photographs.

After induction period of 21 days i.e., on 22nd day and after treatment period of 21 days i.e., on 42nd day the initial and final weight were taken, respectively and blood was withdrawal by retro-orbital plexus method and cholesterol, testosterone level were estimated on 22nd and 42nd day. One rats from each group were sacrificed on 22nd day and 42nd day skin tissue was removed and sent for histopathological studies. The experiment protocol was conducted for 44 days.

Evaluation parameters

Measurement of body weight: The body weights of animals of each group were measured on the 1st day i.e., initial weight and on the 22nd day i.e., intermediate weight and on 42nd day i.e., final weight.

Skin irritation test: Skin irritation test was conducted to evaluate the irritation by the prepared formulations on intact skin of rats. The dorsal area of animal was cleaned with spirit and drug was topically applied to rats. The sites were observed for erythema and edema for 48 h after treatment.

Measurement of T level and cholesterol level: Blood was withdrawal by retro-orbital plexus method and cholesterol, T level was estimated on 22nd (after induction period) and 42nd (after treatment period) day. Samples were sent to AMEY Pathology Laboratory, Socialist Square, Wardha.

Measurement hair length: Hair was plucked randomly by using sterile forceps from the depilated area on 21st day of treatment, hair length was measured by scale and the results were calculated as mean length±SEM of 25 hairs.

Histopathological studies: The skin tissue of each animal from the dorsal area was dissected after removal of hair. The skins were stored in glass container in 10% formalin solution. The samples were sent to histopathology, AMEY Pathology Laboratory, Wardha.

Statistical analysis: Data were analyzed using Graph Pad Prism 8 for Windows (version 8.1.0). Results were expressed as Mean±SEM. Two-way Analysis of Variance (ANOVA) and Bonferroni's post-test were used to test the significance of the difference between the variables in various groups. The p-values of less than 0.05 were considered to be statistically significant.

RESULTS

Effect of ellagic acid on body weight, (on Day 1, 22 and 42): Table 1 shows effect of testosterone on body weight before induction (Day 1st) and after induction (Day 22nd). On 1st day there was no significant difference in body weight of all groups compared to normal. On 22nd day, body weight of rats was significantly increased in control, Group-II, Group-III, Group-IV, Group-V, Group-VI, Group-VIII and Group-VIII when compared with normal group. It shows alopecia was successfully induced in all groups except normal.

Table 1: Effect of ellagic acid on body weight

	Body weight (g)		
Groups	1st day	22nd day	42nd day
Group-I (Normal)	275.15±1.639	303.37±1.655	308.83±1.795
Group-II (Control)	298.37±1.572	332.12±1.925$^{@}$	321.66±1.895$^{@}$
Group-III (Standard)	276.62±1.594	324.95±1.896$^{@}$	304.83±1.497***
Group-IV (Test 1)	273.87±1.493	326.56±1.890$^{@}$	318.15±1.785^{ns}
Group-V (Test 2)	283.85±1.884	323.61±1.906$^{@}$	314.33±1.764^{ns}
Group-VI (Test 3)	288.84±1.648	326.95±1.985$^{@}$	306.83±1.578*
Group-VII (Test 4)	271.58±1.649	335.37±1.832$^{@}$	319.16±1.715^{ns}
Group-VIII (Test1+Std)	285.51±1.819	312.66±1.992$^{@}$	305.37±1.522**

*Each group consist of 6 animals (n = 6), Values expressed as Mean±SEM, 2 way ANOVA followed by Bonferroni test,$^{@}$p<0.0001 compared to normal, *p<0.05, **p<0.01 and ***p<0.001 compared to control, ns: Non significant

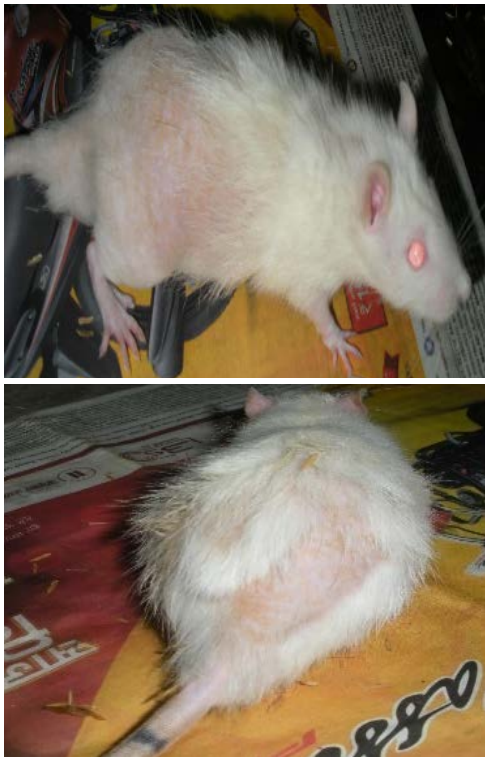

Fig. 2: Observation of skin irritation test

Table 2: Effect of ellagic acid on cholesterol level

	Cholesterol (mg dL^{-1})	
Groups	22nd day	42nd day
I (Normal)	45.195±1.275	50.125±1.250
II (Control)	93.155±1.552@	80.125±1.425@
III (Standard)	90.125±1.568@	63.125±1.360***
IV (Test 1)	88.452±1.705@	85.158±1.405ns
V (Test 2)	92.523±1.632@	81.510±1.402ns
VI (Test 3)	89.025±1.596@	72.465±1.394*
VII (Test 4)	90.450±1.595@	83.588±1.402ns
VIII (Test 1+Std)	91.512±1.782@	69.158±1.376**

*Each group consist of 6 animals (n = 6), Values expressed as Mean±SEM, 2 way ANOVA followed by Bonferroni test, @p<0.0001 compared to normal, *p<0.05, **p<0.01, ***p<0.001 compared to control group, ns: Non significant

Table 3: Effect of ellagic acid on testosterone level

	Testosterone (ng mL^{-1})	
Groups	22nd day	42nd day
I (Normal)	486.21±1.290	493.85±1.302
II (Control)	902.85±1.764@	893.52±1.514@
III (Standard)	898.95±1.685@	740.89±1.423***
IV (Test 1)	890.89±1.898@	854.52±1.499ns
V (Test 2)	885.58±1.648@	812.58±1.472ns
VI (Test 3)	865.89±1.890@	790.85±1.462*
VII (Test 4)	879.56±1.849@	820.15±1.501ns
VIII (Test 1+Std.)	893.20±1.648@	770.85±1.450**

*Each group consist of 6 animals (n = 6), values expressed as Mean±SEM, 2 way ANOVA followed by Bonferroni test, @p<0.0001 compared to normal, *p<0.05, **p<0.01, ***p<0.001 compared to control group

On day 42, body weight in control group was significantly higher (@p<0.0001) compare to normal. Treatment with EA (30 mg kg^{-1}) alone and in combination with standard (EA 10 mg kg^{-1}+Minoxidil 3%) and Minoxidil has significantly decreased the body weight, respectively with p-value (*p<0.05, **p<0.01, ***p<0.001). Treatment with low doses of EA (10 and 20 mg kg^{-1}) decreased body weight, but the results were not statistically significant.

Skin irritation test: Rats were not showed any erythema and/or edema as shown in Fig. 2, this indicates that ellagic acid solution was non-irritant on skin of rats. From findings of skin irritation test it found that ellagic acid is safe for topical use.

Effect of ellagic acid on cholesterol level in alopecia rats: Table 2 shows effect of ellagic acid and in combination with standard cholesterol level (On day 29 and 42). On 22nd day cholesterol level of rats was significantly increased in control Group-II, Group-III, Group-IV, Group-V, Group-VI, Group-VII and Group-VIII when compared with normal group. It shows induction of alopecia was successfully induced in all groups except normal. On day 42, cholesterol level in control group was significantly higher (@p<0.0001) compare to normal. Treatment with EA (30 mg kg^{-1}) alone and in combination with standard (EA 10 mg kg^{-1}+Minoxidil 3%) and Minoxidil has significantly decreased the cholesterol level, respectively with p-value (*p<0.05, **p<0.01, ***p<0.001). Treatment with low doses of EA (10 and 20 mg kg^{-1}) decreased cholesterol level, but the results were not statistically significant.

Effect of ellagic acid on testosterone level in alopecia rats: Table 3 shows effect of ellagic acid and in combination with standard testosterone level (on day 29 and 42). On 22nd day, T level of rats was significantly increased in control Group-II, Group-III, Group-IV, Group-V, Group-VI, Group-VII and Group-VIII when compared with normal group. It shows induction of alopecia was successfully induced in all groups except normal. On day 42, T level in control group was significantly higher (@p<0.0001) compare to normal. Treatment with EA (30 mg kg^{-1}) alone and in combination (EA 10 mg kg^{-1}+Minoxidil 3%) and Minoxidil with standard has significantly decrease the testosterone level, respectively with p-value (*p<0.05, **p<0.01, ***p<0.001). Treatment with low doses of EA (10 and 20 mg kg^{-1}) decrease T level, but the results were not statistically significant.

Fig. 3(a-h): Visual observation in animals of different groups, (a) Normal group, (b) Control group, (c) Minoxidil (3% Topical), (d) EA (10 mg kg^{-1}) (e) EA (20 mg kg^{-1}), (f) EA (30 mg kg^{-1}), (g) EA (topical) and (h) EA (10 mg kg^{-1})+Minoxidil (3%)

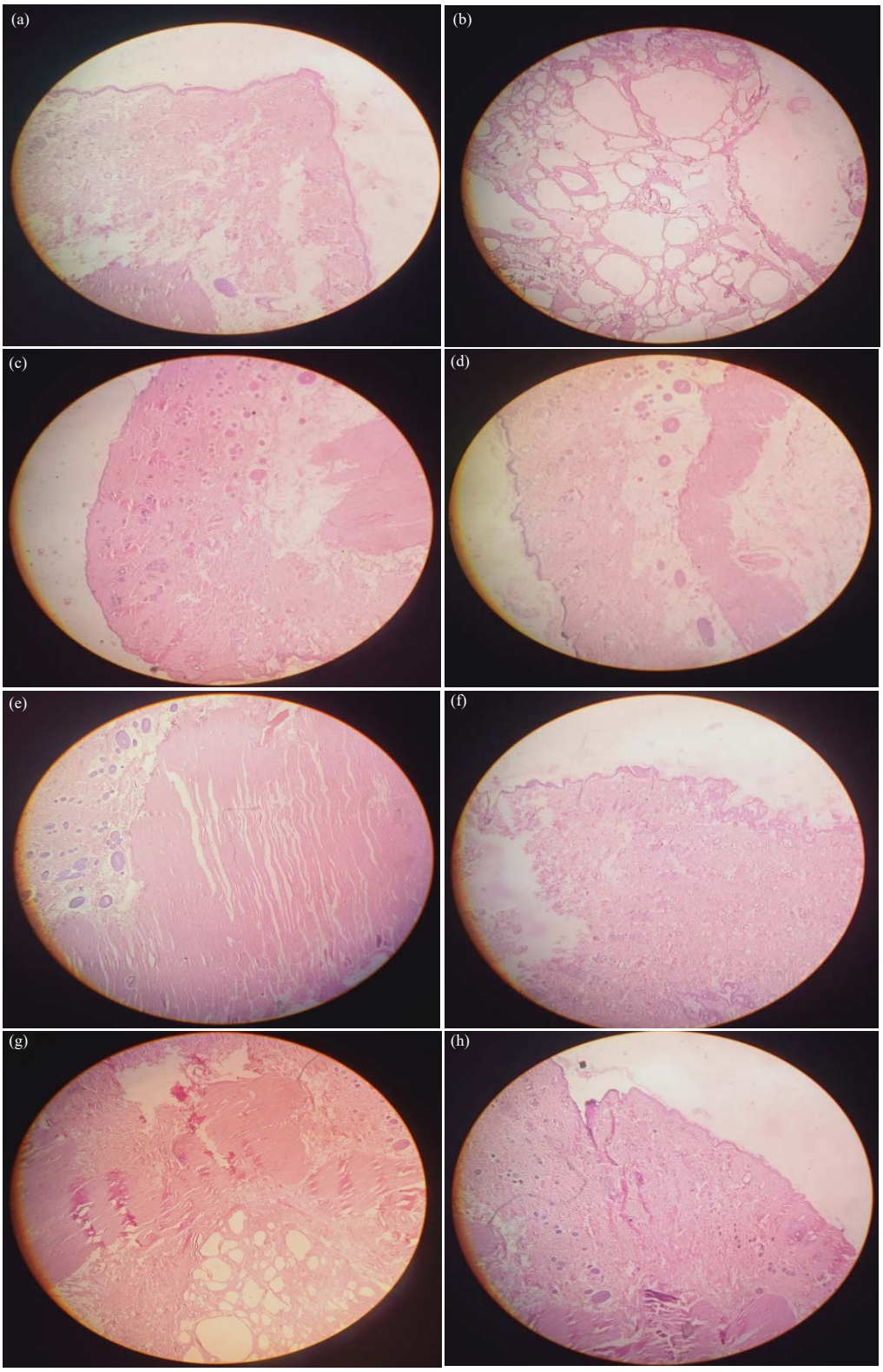

Fig. 4(a-h): Histopathology of skin, (a) Normal rat skin tissue, (b) Control rat skin tissue, (c) Treated with Minoxidil (3%) (Topical), (d) Treated with ellagic acid (10 mg kg^{-1}), (e) Treated with ellagic acid (20 mg kg^{-1}), (f) Treated with ellagic acid (30 mg kg^{-1}), (g) Treated with ellagic acid (Topical) and (h) Treated with ellagic acid (10 mg kg^{-1}) and Minoxidil

Table 4: Observation of hair growth

Groups	Time taken for hair growth initiation (in days) (mm)	Time taken for hair growth completion (in days) (mm)
I (Normal)	2.483±0.1151	2.535±0.1260
II (Control)	0.983±0.2007	2.325±0.2423
III (Standard)	2.433±0.2783	9.532±0.3213
IV (Test 1)	1.239±0.1061	5.302±0.1098
V (Test 2)	1.984±0.1445	6.801±0.1335
VI (Test 3)	2.133±0.2234	7.231±0.2215
VII (Test 4)	1.159±0.1732	5.182±0.3513
VIII (Test1+Std.)	2.398±0.1199	8.231±0.2278

*Each group consist of 6 animals (n = 6), Values expressed as Mean±SEM, 2 way ANOVA followed by Bonferroni test, @p<0.0001 compared to normal, *p<0.05, **p<0.01, ***p<0.001 compared to control group

Measurement hair length: The results shown in Table 4 and Fig. 3, rat treated with EA at different level of produced significant (p<0.01) hair growth.

Histopathology studies: Finding of histopathological studies showed improvement in the histopathological parameters by the treatment of EA alone and in combination with standard (Fig. 4):

- **Normal rat skin tissue:** Section shows no any remarkable changes in epidermis or dermis (Fig. 4a)
- **Control rat skin tissue:** Section shows heavy infiltration of lymphocytes, plasma cells, macrophages in deep dermis. Epidermis shows diffuse atrophy and ballooning degeneration (Fig. 4b)
- **Minoxidil treated rat skin tissue:** Section shows no any remarkable changes in epidermis or dermis. Large amount of hair follicles shows in groups (Fig. 4c)
- **EA (10 mg kg^{-1}) treated rat skin tissue:** Section shows spongiosis diffuse atrophy of squamous epithelium and ballooning degeneration of basal cells of epidermis. Dermal adnexae are within normal layer (Fig. 4d)
- **EA (20 mg kg^{-1}) treated rat skin tissue:** Section shows macrophages in deep dermis and epidermis shows diffuse atrophy (Fig. 4e)
- **EA (30 mg kg^{-1}) treated rat skin tissues:** Section shows hair follicles start to proliferation stages and normal epidermis and dermis cells (Fig. 4f)
- **EA (Topical) treated rat skin tissue:** Section shows infiltration of lymphocytes plasma cells at deep dermis and hydropic changes in epidermis (Fig. 4g)
- **Combination of EA (10 mg kg^{-1}) and Minoxidil (3%) skin rat tissue:** Section shows normal epidermis and dermis cells. Large amount of hair follicles shows in this group (Fig. 4h)

DISCUSSION

Genetic disorder androgenic alopecia has defined pattern which is transferable from one generation to another and it is dependent on androgen. Testosterone and genetic predisposition are needed for androgenic alopecia[32].

Follicles of hairs are targets for miniaturization by androgen which leads to replacement of large and pigmented hairs by depigmented hairs that are barely visible. Therefore, there is progressive reduction in hair density on scalp. Currently androgen action based therapy targeted on dermal papilla at the base of follicle. Dermal papilla are derived from mesenchyme respond to hormones circulating in blood and coordinates remaining follicular cells by paracrine signal it generates[33].

Present study is the first report on hair growth potential of ellagic acid. Current study used rat model of androgenic alopecia for evaluation of hair growth activity of ellagic acid which has been used by Das *et al.*[34] and Wang *et al.*[35].

Testosterone acts either directly or it is converted to dihydrotestosterone by 5α reductase and exerts its effect in hair follicles through binding with androgen receptors[36]. Finasteride, 5α-reductase inhibitor has been utilized by several other researcher for testosterone induced alopecia[37-39]. It is reported by Kim *et al.*[40] that hair loss by testosterone may involve apoptosis of hair follicles rather than androgen metabolic pathway. Present study is an attempt to find alternative to currently used steroid based drugs.

Testosterone significantly increased the body weight, testosterone, cholesterol level and hair loss. Significantly increased testosterone level confirmed induction of alopecia based on data collected at day 22. One animals from each group were sacrificed, skin tissue were removed (stored in 10% formalin) and send for histopathological studies. The histopathological findings on day 22 showed induction of the alopecia by treatment testosterone for 21 days to all groups except normal group. Similar findings in relation with testosterone induced alopecia have been reported by Das *et al.*[34] and Wang *et al.*[35]. Treatment with ellagic acid (30 mg kg^{-1}) alone and in combination with Minoxidil has significantly decreased the body weight. Ellagic acid (30 mg kg^{-1}) alone and in combination with standard Minoxidil has significantly decreased the testosterone and cholesterol levels. Histopathological findings on day 42nd reduced symptoms of androgenic alopecia by the treatment of ellagic acid alone and in combination with standard in sub-effective doses. Result of the present study revealed potential effects of ellagic acid alone and in combination with standard on various parameters of hair growth promoters viz.,

skin irritation test, hair length, testosterone, cholesterol level and skin biopsy. Alopecia was not present in animals that were treated with ellagic acid along with testosterone. Besides biochemical parameters, histopathological data also suggested attenuation of androgenic alopecia by gallic acid. Thus, ellagic acid is potent drug for oral and topical use in commercial formulations for androgenic alopecia. Ellagic acid has been reported to have antioxidant[41]. Therefore, it could be suggested that antioxidant property might have contributed in hair growth potential of this plant. Ammar *et al.*[42] has reported that plant pomegranate which contains ellagic possess antiandrogenic property against benign prostate hyperplasia which points towards the ellagic acid against androgenic alopecia. Similarly Nam *et al.*[43] reported that *Trapa japonica* has also been to have hair growth promoting activity. This preclinical study opens concept for hair growing potential of ellagic acid. Further studies investigation on antiandrogenic mechanism of ellagic acid as well as clinical studies will be addition to this study.

CONCLUSION

Results of present study discovered the potent of ellagic acid alone and possible synergistic effect of ellagic acid and Minoxidil combination that can be beneficial for the testosterone-induced alopecia in rats.

The EA showed significant decrease in body weight, testosterone and cholesterol level as compared to the control group. Finding of histopathological studies supported the fact that ellagic acid have excellent hair growth promoting activity by an enlargement of follicular size and a prolongation of the anagen phase.

SIGNIFICANCE STATEMENT

This study discovers the possible use of ellagic acid in treatments of androgenic alopecia. This study will help the researcher to uncover the role of ellagic acid and combination of ellagic acid with Minoxidil for treatment of alopecia. Consequently present study will help the researcher to find important novel ways of treating androgenic alopecia that large numbers of researchers were not able to explore. Thus, a new theory on treatment of androgenic alopecia with minimal or no side effects may be arrived at the horizon.

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