RESEARCH ARTICLE

Effect of *Convolvulus arvensis* Ethanolic Extract on Testosterone-induced Alopecia in Mice

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ABSTRACT

Background and Objective: Androgenetic alopecia is an age-related disease. It's the most prevalent type of non-scarring progressive hair loss, affecting men and women. Here, *Convolvulus arvensis* ethanolic extract is investigated for its growth-promoting effects in albino mice with testosterone-induced hair loss.

Material and Method: 50 adult male albino mice (2-3 month) divided into 5 group 10 mice in each group (I) intact negative control (II) Testosterone gel 1% only (induction group) (III) Testosterone gel 1% + finasteride solution 2% (standard group) (IV) testosterone gel 1% + *Convolvulus arvensis* cream 3% (extract group) (V) testosterone gel 1% + glycerine cream 15% (vehicle group) testosterone gel 1% was applied topically to the back of the mice to all group except negative control group (I) for 21 days to induce hair loss hair growth promoting effect evaluated by visual observation, histopathological study of follicular density and anagen/telogen ratio and testosterone, dihydrotestosterone serum level.

Result: animals in extract group showed less hair loss as compared to those treated with induction and vehicle groups that possess A patch of hair loss, *Convolvulus arvensis* ethanolic extract increase follicular density and anagen/telogen ratio significantly compared to induction group, these result suggest that extract promotes hair growth and prevent hair loss by inducing the anagen phase in resting hair follicles and might therefore be a potential hair growth-promoting agent.

Keywords: Androgenetic alopecia, *Convolvulus arvensis*, Hair growth, HPLC, Testosterone.

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INTRODUCTION

Androgenetic alopecia (AGA) is the most prevalent form of progressive hair loss disorder that affects both men and women after puberty. It is an age-related disease characterized by non-scarring progressive alopecia due to follicular miniaturization and distinct pattern distribution. A major role is played by genetic factors and the peripheral action of androgens, which trigger the gradual conversion of terminal hairs to vellus hairs.[1] Male Androgenetic alopecia has been related to several other medical conditions (comorbidities), including: coronary heart disease, prostate enlargement and metabolic syndrome (Abdominal obesity, Hypertension, Dyslipidemia, diabetes, insulin resistance).[2-5] Men infected with SARS-CoV-2 have an increased risk of severe COVID-19 disease compared to women, McCoy *et al.* show that 79% of hospitalized men with COVID-19 presented with AGA.[6]

In women, Androgenic alopecia is associated with a greater risk of polycystic ovary syndrome (PCOS) this provides a potential link with insulin resistance, obesity, and metabolic syndrome.[7]

Testosterone is converted to the more potent dihydrotestosterone by 5α reductase enzyme. Skin cells contain 5α reductase (types I and II). Sebaceous glands have type I enzyme, while hair follicles and the prostate gland have type II enzyme. Testosterone and dihydrotestosterone act on androgen receptors in the dermal papilla[8] dihydrotestosterone binds to the follicle androgen receptor and activates the transformation of large, terminal follicles to miniaturized ones. The duration of anagen shortens with successive hair cycles, and the follicles become smaller, producing shorter, finer hairs.

Chronic inflammation and the interaction between dermal papilla and follicular epithelium have been associated with the progression of AGA. In patients with AGA, histopathological studies of the scalp typically reveal peribulbar inflammation (lymphocytes) and perifollicular fibrosis.[9]

The Food and drug administration (FDA) has approved minoxidil and finasteride for the treatment of AGA. The treatment aims to retain current hair density, which is why it's important to get started as soon as possible.[10]

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Natural products have been widely advocated in the hair care industry, and the search for natural remedies is continuously promoted. Herbs such as *Hibiscus rosa-sinensis*,[11] *Cuscuta reflexa*,[12] *Capillus veneris Linn*,[13] *Asiasari radix*,[14] *Thujae occidentalis*[15] are used as hair growth promoters.

C. arvensis (field bindweed) is a species of bindweed of the genus *Convolvulus* were grown in Iraq. Aerial parts of *C. arvensis* have pharmacological effect antioxidant, immunostimulant effect, vasodilating effect, cytotoxic effect, hepatoprotective effect, antibacterial effect, and antidiarrhoeal effect, in this study, we will examine the effect of it on hair growth.[16]

MATERIALS AND METHODS

Plant Material and Extraction

The aerial part *C. arvensis* were collected from Al Musayeb, city south of Baghdad. Authentication of the plant carried out by National Herbarium in Botany directorate at Abu-Ghraib. The aerial parts were rained with tap water, dried at room temperature in the shade. Then ground as powder and weighting. The extraction was done at Mustansiriyah University Pharmacognosy and Medicinal Plants department, College of Pharmacy (Iraq). The powdered aerial parts of *Convolvulus arvensis* (100 gm) are defatted with hexane (700 mL) , the defatted plant material is further extracting with 80% ethanol (700 mL) using soxhlet extractor, the ethanolic extract is concentrated by evaporation under reduced pressure using a rotary evaporator to get a dry dark brown extract, resulting residue is the crude polyphenol extract. Preliminary phytochemical screening for flavonoid, tannins, saponin, alkaloid and terpnoid was done.

Chromatogram by HPLC for Identification of Active Constituent

The extract is analyzed by (HPLC) to investigate the presence of flavonoids, the identification is make by comparing the retention times obtain at the specific chromatographic condition of the analyzed samples and veritable standards.

One gram of dry sample was crushed to small pieces in pastel-mortar followed by suspending fine crushed sample into 200 mL of HPLC grade methanol and deionized water (80:20 mv/v), the sample were shaking. The extraction of flavonoid was subjected to ultra-sonication (Bransonifier, USA) at 60% duty cycles for 25 min at 25°C followed by centrifugation at 7500 rpm for 15 min. the clear supernatant of each sample was subjected to vacuum-assisted evaporation (BuchiRotavapor Re Type). The dried samples were re-suspended in 1.0-mL HPLC grade methanol by vortexing, the mixture was passed through a 2.5 µm disposable filter, and stored at 4°C for subsequent analysis, after which 20 µL of the sample was injected into the HPLC system according to the optimum separation conditions.

Table 1: Phytochemical Screenning of *C. arvensis* Ethanolic extract

Test	*Result*
Flavonoid	+
Terpenoid	+
Saponin	+
Tannins	+
Alkaloid	+

+, - represent the presence and absence of Phyto-constituents, respectively.

Animal

Adult healthy male albino mice (weight: 25–30 g and age: 8–12 week) were obtained from the animal house of Iraqi Center for Cancer and Medical Genetics Research, University of Al Mustansiriya, Iraq.

Mice were housed in the animal house of the Iraqi Center for Cancer and Medical Genetics Research, University of Al Mustansiriya in polypropylene cages and in an environmentally controlled condition (22 ± 25°C, relative humidity of 50 ± 5%) with a photoperiod 12 hours light/dark cycle and allowed free access to water and food. The Institute Review Board (IRB) Al-Nahrain University College of Medicine approved the current research protocol.

Testosterone Gel

Marketing preparation testogel® 1% (Laboratoros Rubio) transdermal gel provide 50 mg/5 g in each sachet used topically

Finasteride Solution

Two grams of finasteride powder (Xian Prius Biological Engineering Co., Ltd China) dissolved in vehicle of (ethanol/propylene glycol, 90:10).

Extract Cream

3 g of extract dissolve it in 6 mL of ethanol then complete the weight to 100 g with glycerine cream 15% (Aquasoft) as a vehicle to obtain 3% extraction cream.[20]

Induction of Alopecia

Depilatory cream was used to remove the dorsal hair of mice (2 x 2.5 cm). The mice model of AGA was established according to a previous experiment by topical application of Testosterone gel 1%, Testosterone gel 1% applied on the dorsal area on the shaved back skins of mice for one hour on basis of daily use for 21 days.[21]

Experimental Design

The animals divided into 5 groups of 10 albino mice in each group randomly as followings:

Group (I) apparently healthy mice as a negative control (no induction, no treatment), group (II) testosterone gel 1% only as induction group, group (III) testosterone gel 1% + finasteride solution 2% as a standard group, group (IV) testosterone gel 1% + *C. arvensis* ethanolic extract cream 3% as extract group, group (V) testosterone gel 1% + glycerine cream 15% once daily as vehicle group. Animals in all group except group (I) received testosterone gel 1% applied topically for 1 hours and then apply the treatment once daily for 21 days.

Determination of Serum Hormones Concentration

To determine effect of *C. arvensis* ethanolic extract on serum level of testosterone and dihydrotestosterone in mouse model

of testosterone induced hair loss, blood was collected at day 22 from mice of all groups (1-mL of blood from heart puncture in gel tube from mouse. After that, allow the samples to clot at room temperature for 1 hours Then, the serum was separated by centrifugation at 3,000 RPM for 20 minutes). The concentrations of hormones were determined using ELISA kits according to the manufacturer's instructions.

Histopathological Study

On day 22, animals in all groups were euthnized, full-thickness skin samples were cut using a sterile sharp blade and immersed in the bottom of a 10 mL sterile test tube filled with ten times its volume with 10% neutral buffered formalin and then stained with hematoxylin and eosin. Observed two parameter

- Follicular Density (number of hair follicle/mm)
- follicle number in active phase (anagen) and in resting phase (telogen), Anagen/telogen ratio

Qualitative Evaluation

Photographs were taken in day 21 for evaluation the difference in hair growth between groups.

Statistical Analysis

The mean ± SD (Standard Deviation) of the data are presented. Using the program graph pad prism version 9.1.2, data was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD as a post hoc test. $p < 0.05$ was regarded a significant level.

RESULT

HPLC Analysis of *C. arvensis* Ethanolic Extract

Quantitative estimations of active constituents were done by using HPLC in which identifications were made by comprise of retention times obtained at identical chromatographic conditions of analyzed samples the results show the presence of (vitexin, luteolin, nargenin, p-comarin, Rutin, Kaempferol and Quercetin) in comparison with retention time of standard flavonoids as shown in Table (2 and 3) the flavonoids (Rutin and quercetin) found in high concentration, we found in the phytochemical analyses of our formulation may offer more specific support for the wellbeing of hair physiology. Quercetin keep the hair follicle in the anagen phase longer by inhibiting 5αR type 2 and preventing DHT formation[17] Quercetin is an herbal bioactive flavonoid commonly used for the treatment of metabolic and inflammatory disorders reducing the levels of testosterone.[18]

One of the most significant phytochemicals is natural rutin. Rutin is a bioflavonoid that has antioxidant properties. Rutin has a wide range of scavenging properties on oxidizing species like hydroxy radicals, superoxide anions, singlet oxygen, and hydroxyl radicals by donating hydrogen atoms to peroxy radicals, superoxide anions, and singlet oxygen and hydroxyl radicals. It also acts as a terminator and chelator of metal ions that can oxidize lipid peroxidation.[19]

Serum Hormones Concentration

Hormones serum level were detected by standard diagnostic test ELISA kits, Table 4 show effect of *C. arvensis* ethanolic

Table 2: HPLC Analysis of Standard

Subject	*Retention time*	*Area*	*Concentration each 25 µg/mL*
Vitexin	1.55	366292	12.0688
Rutin	3.13	427756	15.3513
Quercetin	4.37	375200	13.5811
Kaempferol	5.55	351536	12.6159
Luteolin	6.64	404714	14.5244
Narngenin	7.79	470392	16.8814
P-comarin	8.97	419558	15.0571

Table 3: HPLC Analysis of *C. arvensis* ethanolic extract

Subject	*Retention time*	*Area*	*Concentration each 25 µg/mL*
Vitexin	1.61	119695	3.5664
Rutin	3.17	1055116	31.4377
Quercetin	4.45	685091	20.4126
Kaempferol	5.55	325409	9.6957
Luteolin	6.62	234112	6.9755
Narngenin	7.71	234607	6.9902
P-comarin	8.98	330644	9.8517

Table 4: Serum Hormones Concentration

Group	*Serum T*	*Serum DHT*
Negative control (No induction, no treatment)	624.2 ± 208.4	73.48 ± 12.22
Induction (Testo gel 1% only)	1181 ± 278.9*	104.9 ± 24.18*
Standard (Testo gel 1%+ finasteride solution 2%)	873.3 ± 366.6	83.06 ± 17.01
Extract (Testo gel 1% + *Convolvulus arevensis* ethanolic extract cream 3%)	818.4 ± 352.8	103.4 ± 12.43*
Vehicle (Testo gel 1% + glycerine cream 15%)	1098 ± 258.4*	105.6 ± 15.58*

Values represent mean ± SD
*$p < 0.005$, significance *vs* control

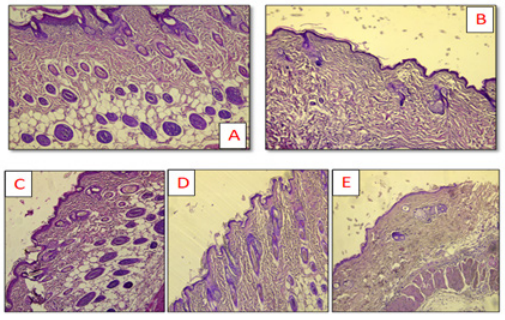

Figure 1: Histology Skin section of Mice (10X H&E stain) (A)Control Group (no induction, no treatment) (B) treated with Testosterone gel 1% only (C) Treated with Testosterone gel 1%+ finasteride solution 2% (D) Treated with Testo gel 1%+ Convolvulus arvensis ethanolic extract cream 3% (E) Treated with Testo gel 1%+ glycerine cream 15% (Vehicle).

Table 3: Follicular density and anagen/telogen ratio in sections of skin of different groups of study animals.

Group (Treatment)	*Follicular Density (no./mm) mean ± SD (n=10)*	*Anagen/telogen ratio*
Negative control (No induction, no treatment)	5.100 ± 0.8756	2.250 ± 1.161
Induction (Testo gel 1% only)	2.400 ± 0.5164*	0.4500 ± 0.6433*
Standard (Testo gel 1%+ finasteride solution 2%)	3.600 ± 0.9661**	2.000 ± 1.080**
Treated (Testo gel 1% + *Convolvulus arevensis* ethanolic extract cream 3%)	3.500 ± 0.7071**	1.883 ± 0.8098**
Vehicle (Testo gel 1% + glycerine cream 15%)	2.600 ± 0.8433*	0.5330 ± 0.6026*

Values represent mean ± SD
*p <0.005, significance *vs* control
**p <0.05, , significance *vs* Induction

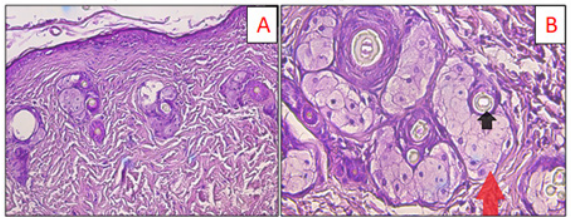

Figure 2: Histology Skin section of Mice H&E Stain, Treated with Testo gel 1% only showing miniaturization of Hair follicle and vellus hair (black arrow) hyperplasia of sebaceous gland (red arrow) (A) 10X (B)40X.

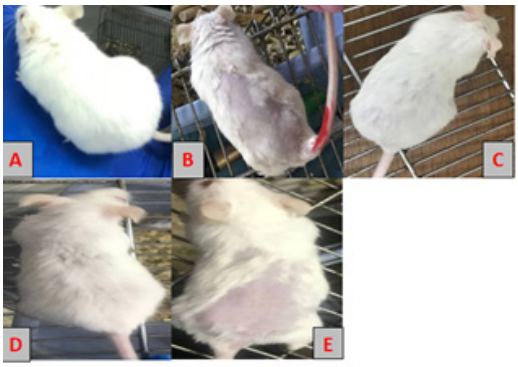

Figure 3: Comparison of Hair growth/loss by visual observation after 21 days (A) Negative Control Group (B) Induction Group (C) Standard Group (D) Extract Group (E) Vehicle Group.

extract on serum hormones level on day 22 of treatment, induction and vehicle groups have been shown to increase level of serum T and DHT significantly compared to negative control group , standard and treated groups decrease level of serum T and DHT but the result were not statistically significant

Histopathological Study

The morphology of the hair follicle seen in the microscope obtained from a longitudinal section of the dorsal skin. Mice in the normal group had more hair follicles, while in the induction group and vehicle group, hair follicles were sparse and miniaturization is shown in Figure 2. The follicles appeared to be bulbous. Topical finasteride and Convolvulus arevensis were used to prevent testosterone's effect on hair follicle miniaturization in the III and IV animal groups, respectively Figure 1. The hair follicle in T and V group was sparse and the follicular density was significantly less than the normal group. This effect blocked by finasteride and plant extract, a number of hair follicle/mm significantly increased compared to the induction group.

Qualitative Evaluation

After 21 days of treatment, the observations were taken. According to the visual observations, the animals in the Extract group had less hair loss than those in the induction and vehicle groups, which had a patch of dispersed hair loss. The extract group's hair growth pattern is less than that of the standard drug finasteride. The extract's ability to promote hair growth has been assessed in these investigations. The extract was found to have good activity against testosterone induced alopecia.

DISCUSSION

Androgenetic alopecia is a heritable, androgen-dependent condition that manifests itself in a predictable fashion. Androgenetic alopecia requires testosterone as well as a genetic susceptibility. Androgen-stimulated hair follicle miniaturization occurs, resulting in the replacement of large, pigmented hairs (terminal hairs) with scarcely visible, depigmented hairs (vellus hairs). As a result, visible scalp hair density decreases with time.[13]

The only approved medications for the treatment of androgenetic alopecia are oral finasteride and topical minoxidil. Topical minoxidil shortens telogen, forcing resting hair follicles (HFs) to enter the anagen phase prematurely. Finasteride is a type II 5α reductase inhibitor that prevents testosterone from being converted to DHT. Even at therapeutic doses, both medications have few side effects, which cannot be overlooked given the requirement for long-term treatment. Contact dermatitis, facial hypertrichosis, and transient hair shedding are among side effects of minoxidil. Telogen effluvium has also been documented following therapy discontinuation. Erectile dysfunction is a significant side effect of finasteride, with an absolute risk of roughly 1.5%. Even after therapy, this side effect might last for years, causing anxiety and mood swings and lowering the patient's quality of life.[22]

Increased dihydrotestosterone levels in hair follicles promote hair loss because dihydrotestosterone binds to the androgen receptor more easily than testosterone in sensitive hair follicles' dermal papilla cells (DPCs). The 5α reductase enzyme, on the other hand, is a crucial enzyme in the conversion of testosterone to dihydrotestosterone. To prevent hair loss, the enzyme 5α reductase was suppressed. Finasteride was chosen as the standard.[23]

C. arvensis phytochemical screening revealed the presence of a variety of chemicals in this plant, including terpenes, flavonoids, and tannins.[25] *C. arvensis* may have antioxidant properties due to the presence of flavonoids (quercetin and

rutin). Kim *et al.,* on the other hand, found that testosterone can cause hair loss through apoptosis of hair follicles rather than the androgen metabolic pathway.[26] As a result, it's possible that flavonoids have a role in this plant's ability to grow hair.

Quercetin, a polyphenolic flavonoid phenylbenzopyrone found in plants, is well-known for its antioxidant properties. Quercetin also possesses anti-inflammatory properties, reduces lipid peroxidation, and works as an antihistamine. 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 allow for topical absorption and availability of HFs at the appropriate site. In this study, we used visual observation, follicular density, and the anagen: telogen ratio to assess the effect of ethanolic extract on testosterone-induced hair loss. The results demonstrated that finasteride and ethanolic plant extract inhibit testosterone's effect on hair follicles.

CONCLUSION

The present study discovered the effect of *C. arvensis* ethanolic extract for hair growth promoting effect on testosterone-induced alopecia in mice.

C. arvensis ethanolic extract showed the active flavonoid constituent by HPLC (quercetin and rutin) and significant increase in follicular density and anagen/telogen ratio compared to induction group.

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