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Effect of Capparis spinosa Ethanolic Extract on Testosterone induced alopecia in Mice

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Abstract

Androgenetic alopecia (AGA) One of the most prevalent chronic disease observed in dermatologic outpatient departments is male or female pattern hair loss. a disorder that affects more than 80% and 42% of Caucasian men and women over the age of 70 years, respectively. The goal of this study was to see how effective a preparation was at promoting hair growth of the Ethanolic extract of Capparis spinosa on albino mice using a topical testosterone gel- induced alopecia model. Material and Methods : The albino mice were divided into 5 groups 10 mice in each group.:(A) intact negative control (without testosterone) (B) Testosterone gel 1%only (C) Testosterone gel 1% + Finasteride solution (2%) (D) Testosterone gel 1% + Capparis spinosa cream(10%) (E) Testosterone gel 1% + glycerin cream 15% (vehicle). Topical Testosterone gel (1%) was used to induce alopecia in all intervention groups except negative control group , C.spinosa Cream 10% was applied topically to the back skin of animals in the respective group. Hair growth was assessed using visual observation and histological investigation of multiple skin slices using parameters such as (anagen/telogen ratio , follicle density (number of follicles/mm) , Standard diagnostic test ELISA kits were used to detect testosterone and dihydrotestosterone levels in the serum, according to the manufacturer's recommendations. . Those treated with testosterone showed a patch of diffuse hair loss after 21 days, whereas animals treated with C. spinosa demonstrated less hair loss than others who were only given testosterone. Results: C. Spinosa treated group had a follicular density of 5.6 ± 1.577, compared to 2.4 ± 0.516 in the testosterone group and 3.6 ± 0.966 in the finasteride group .Anagen/telogen ratio was significantly affected by C.spinosa which was 3.3 ± 1.475 as compared with 0.5 ± 0.408 and 2 ± 1.080 for Testosterone and Finasteride groups respectively. Testosterone and dihydrotestosterone serum levels was significantly affected by C. spinosa as compared with testosterone and finasteride treated groups. C.spinosa was discovered to have good action against Testosterone-induced alopecia based on visual observation and quantitative data (follicular density, anagen/telogen ratio, and serum hormones concentration). Conclusion: C.spinosa, when applied topically, promotes hair growth development and has an anti androgenic action, making it a promising therapy option for male pattern baldness and other androgen dependent condition .

Keywords

Androgenetic alopecia, Capparis spinosa, testosterone, dihydrotestosterone , hair growth, androgen

Alopecia is a common issue in the cosmetics industry as well as primary health care. It's a dermatological condition that's been known for over a thousand years. It can be found all over the world, and it affects 0.2-2 %of the global population. Some of the causes include nutritional deficiency, aging, hormone imbalance, genetic tendencies, acute illness, exposure 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, and other fungal infections. Hair loss can occur in both men and women as a result of surgery and acute stress (1) .

Androgenetic alopecia (AGA) the most prevalent chronic disease observed in dermatologic outpatient departments is male or female pattern hair loss. It's an age-related disease that affects more than 80% and 42% of Caucasian men and women over 70 years, respectively (2) . The Asian population has a lower prevalence of AGA, AGA was observed in 14.1% among all Korean men of all ages according to a study, the frequency of AGA in the African population is 14.6 % in males and 3.5% in women. (3).

Androgenetic alopecia (AGA) There are two parts to the phrase androgen (andro): androgen and genes (genetic). It refers to the systematic loss of scalp hair in men and women who are genetically prone to it, and it is the most common type of hair loss. In this situation, their 5alpha-reductase enzyme activity and dihydrotestosterone (DHT) levels increased. Androgens are regarded to be one of the most common causes of alopecia, in addition to a variety of inherited and environmental variables. Over time, androgen causes normal-sized scalp hair follicles to miniaturize, resulting in miniaturizing hair follicles (4). In the case of AGA, the goal of treatment is to prevent hair miniaturization and regulate hair loss. Despite the fact that minoxidil and finasteride are the only two FDA-approved drugs for hair loss treatment, alternative non-surgical therapies such as dutasteride, spironolactone, platelet rich plasma (PRP), microneedling (MN), and low-level laser therapy have been used (LLLT) (5). AGA requires long-term treatment, and the typical FDA-approved medications possess side effects and toxicities When compared to conventionally marketed medications, herbal sources can provide beneficial treatment with less adverse effects (6). **Capparis spinosa** is a Mediterranean plant that grew in the dry regions of western or central Asia. Capers, which are members of the Capparaceae family, are rich in polyphenols such as glucosinolates, alkaloids, phenols, and flavonoids (7) which is responsible for decreasing lipids, treating diabetes ,antioxidant, anti-inflammatory, antibacterial as well as effect on hormone change so suggest management of AGA(8)

Material and methods

plant material

Plant collection :the aerial part of C.spinosa were collected from Almusayeb city south of Baghdad . Authentication of the plant carried out by national herbarium in Botancy directorate at Abu-Ghraib.

Extraction of plant materials: The aerial parts were dried at room temperature in the shade. Then grinded as powder and weighting. Then powdered aerial parts of C.spinsa (100 gm) are defatted with hexane (700ml) , then defatted plant material is further extracting with 80% ethanol (700ml) using soxhlet extractor, the ethanolic extract is concentrating by evaporation under reduced pressure using rotary evaporator to get a dry dark brown extract .

Preparation of topical cream of crude Capparis spinosa extract 10%

Firstly 10 g of crude extract weighted and putted in beaker and dissolve it in 6ml of ethanol and stirring it until it dissolve completely then complete the weight to 100g with glycerine cream 15% (Vehicle) and mix the combination for 5 minute by spatula and then put the cream in small plastic container.

Preparation of standard Finasteride 2% solution

Weighing (2 g) of pure Finasteride powder by using electronic balance in a beaker and dissolved in 90ml of ethanol then add 10 ml propylene glycol (ethanol/propylene glycol, 90:10) to get the concentration 2% and placed on magnetic stirrer with magnetic bar and stirred for 15 minute to get homogenous solution stored in dark glass test tubes with screw caps and in dark place at room temperature(9).

Experimental animal

Well-being male adult albino mice (weight: 25-30 g and age: 8-12-week) obtained from the animal house of Iraqi Center for Cancer Research /University of Al Mustansiriya and Vaccine and serum Institute Mice were kept in the animal house of the Iraqi Center for Cancer Research /University of Al Mustansiriya in polypropylene cages and in an environmentally controlled condition (22 ± 25 ˚Celsius), Allowable free access to water and food with a relative humidity of (50%) and a photoperiod of (12) hours of light/dark. Animal suffering was minimized, and the number of animals utilized in the studies was reduced to a minimum. According to university rules, the study was authorized by the Institute Review Board (IRB) of Al-Nahrain University College of Medicine.

Study design

Induction of alopecia

The use of testosterone as an applied topically greatly reduced hair growth. Depilatory cream will be used to carefully remove mice's dorsal hair. Testosterone gel 1% applied on the dorsal area on the

shaved back skins of mice one time daily for 3 weeks(10).

Treatment

After shaving of back mice hair with depilatory cream, daily application of Testosterone gel 1% on the skin of mouse back to induce alopecia to all group except group (A) (control group) once daily for 21 days . The animals were randomly separated into five groups, each with ten albino mice. The method reported by Matias et al (11) will be used with slight changes. A similar technique was used by Pandit et al (12).

Group (A). apparently healthy mice (as a negative control group) .

Group (B). alopecia was induced by testosterone gel (1%)without treatment (Induced group).

Group(C). alopecia induced by testosterone gel(1%) applied topically and then after one hour apply standard treatment Finasteride solution(2%) as (standard group).

Group (D). alopecia induced by testosterone gel 1% applied topically and then after one hour apply C.spinosa crude extract cream (10%) (treatment group).

Group(E). alopecia induced by testosterone gel 1% applied topically and then after 1 hour apply glycerine cream (15%) as (vehicle group).

Quantitative evaluation

Blood sample collection

At day 22, the mice were anesthetized by using a slice of cotton socked through chloroform and placed the mouse in a closed glass jar for limited minutes to be anesthetized by inhalation, after which the blood was collected (1 ml of blood from heart puncture in gel tube from mouse. After that, leave the samples to clot for 1 hour at room temperature. The serum was then separated by centrifugation at 3,000 RPM for 20 minutes to assess testosterone and dihydrotestosterone (DHT) levels in serum using standard diagnostic test ELISA kits, as directed by the manufacturer.

Histopathological sample preparation

A histological study of all tissues was carried out in histopathology department /Ibn Sina University of Medical and Pharmaceutical Sciences to observe the changes in tissues .After the euthanasia of mice. The skin lesions collected, kept in phosphate-buffered formalin (Solution of 10% Formalin is prepared by adding (2700 ml) distilled water to (300 ml) formaldehyde for paraffin sectioning (13).

Qualitative assessment of hair growth

After 21 days, the variance in hair development in every group was assessed visually and documented with photographs.

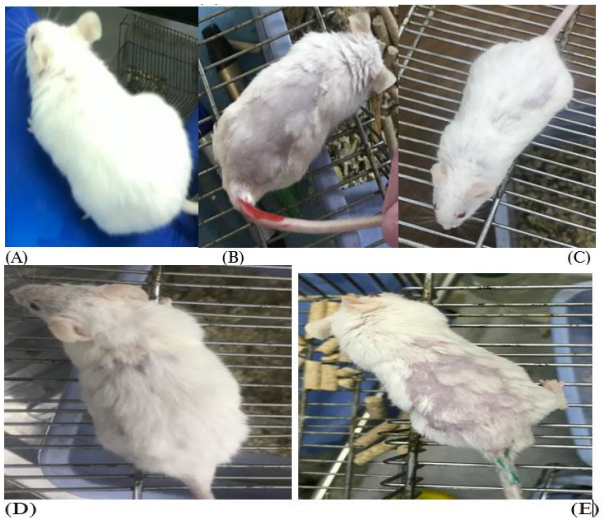
Statistical analysis

Numeric variables were expressed as mean ± standard division and all statistical comparisons were done by use of independent t-test and one way ANOVA t test with P ≤0.05 was considered statistically significant.

Results

Qualitative Evaluation

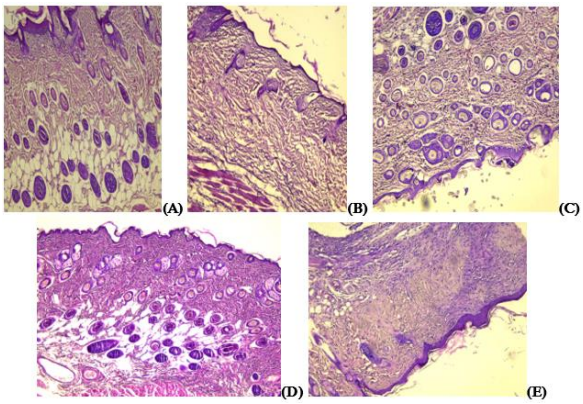
The animals in groups (B) and (E) had alopecia diffuse. After 21 days of therapy with 1% testosterone gel, hair loss from the dorsal area of mice was obviously visible (Figure. 1). The ethanolic extract of crude Capparis spinosa cream (10%) was given to the animals in group (D), along with testosterone gel (1%). The alopecia condition was not observable in this group of mice, indicating that the C.spinosa extract inhibited testosterone activity and hair loss caused by testosterone. The results were the same as C. spinosa extract in animals in group (C) who were given Finasteride and testosterone at the same time.



(Figure. 1) (Visual observation) Comparison of hair growth/ loss in each group by visual observation after 21 days: (A) control animal. (B) Animals treated with Testosterone showing hair loss. (C) Animals treated with Testosterone and Finasteride showing normal hair growth development. (D) Animals treated with Testosterone and ethanolic extract of Capparis spinosa showing normal hair growth.(E) Animals treated with Testosterone and vehicle showing diffuse hair alopecia

Quantitative Evaluation

The testosterone treatment group produced hair follicle shrinking, according to microscopic examination of skin slices from group (B) animals. The follicles were short and bulbous in appearance. Many hair follicles were in the telogen phase, which resulted in a reduction in the number of hair follicles (Figur. 2). The effect of testosterone on hair follicle miniaturization was inhibited in both the ethanolic extract of Capparis spinos cream (10%) treated group and the topical Finasteride treated group. The length and quantity of hair follicles were also increased, according to histology findings. The number of follicles in the anagen phase increased as the period of treatment with Capparis spinosa ethanolic extract increased. The follicular density detected with C.spinosa- treated animals was 5.6 ± 1.577, whereas it was 2.4 ± 0.516 in testosterone-treated animals and 3.6 ± 0.966 in Finasteride-treated animals (Table 1).



(Figure.2) Histology of skin slices (H&E X 10 stain): (A) Skin of a negative control animal . (B) Testosterone-treated animal's skin. (E) Testosterone gel 1% and vehicle-treated animal skin.

(C) Animal skin given testosterone and finasteride 2%treatment. (D) Skin of an animal given testosterone gel 1% and a 10% ethanolic Capparis spinosa extract cream.

Table (1) Follicular density and anagen/telogen ratio in sections of skin of different groups of animals.

Groups	Follicular density (no./mm), Mean ± SD (n = 10)	Anagen/telogen ratio Mean ± SD (n = 10)
(A)Control	5.1 ± 0.875	2.25 ± 1.160
(B)Testosterone gel1%	2.4 ± 0.516*	0.48 ± 0.579*
(C)Finasteride +testosterone gel1%	3.6±0.966**	2 ± 1.080**
(D)C.spinosa cream(10%)+ testosterone gel 1%	5.6 ±1.577**	3.3 ± 1.475**
(E)Vehicle+ testosterone gel 1%	2.2 ± 0788 *	0.5 ± 0.408*

*p<0.005, significance versus negative Control group
**p<0.05, significance versus Induced group

Table (2) Effect of the different groups on serum Testosterone and Dihydrotestosterone levels on day 22:

Groups	Testosterone Mean ± SD (n = 10)	Dihydrtestosterone Mean ± SD (n = 10)
(A)Control	624.16±208.410	73.47 ± 12.214
(B)Testosterone gel1%	1039.47±374.551*	132.49± 68.865 *
(C)Finasteride 2%+testosterone gel 1%	999.67±371.984	78.42± 13.025**
(D)C.Spinosa cream(10%)+ testosterone gel 1%	513.39±123.544	49.64± 13.668 **
(E)Vehicle+ testosterone gel 1%	1098.27±285.405*	105.58± 15.579*

*p<0.005, significance versus Control
**p<0.05, significance versus Induction

Effects of ethanolic extract of C.spinosa in regards on serum hormones levels ,The levels of testosterone and dihydrotestosterone in serum were evaluated using standard diagnostic test ELISA kits to investigate the effect of 5 alpha-Reductase enzyme inhibitory on hormone metabolism. (Table

2) Revealed the levels of testosterone and dihydrotestosterone in serum were reduced significantly in C.spinosa treated group compared with testosterone induced group and vehicle treated group,(P≤0.05). The results indicated that animals receiving ethanolic extract of C.spinosa cream treatment affect serum testosterone and dihydrotestosterone levels. The Finasteride treated group significantly reduce Dihydrotestosterone level in serum compared with Testosterone induced group and vehicle treated group,(P≤0.05).

Discussion

Alopecia was induced in mice in the current study by administering topical testosterone. Conversion of Testosterone to Dihydrotestosterone, a more strong androgen, causes hair follicle miniaturizing that shift in hair growth cycle's phase, resulting in androgenic alopecia , in the conversion of testosterone to dihydrotestosterone, the enzyme 5alpha-reductase type 2 is involved. Finastride is a synthetic anti-androgenic medicine used to promote hair growth by inhibiting the enzyme 5alpha-reductase, which converts testosterone to the more potent androgen dihydrotestosterone (12). When the mice were given finasteride at the same time that they were given testosterone, the alopecia was prevented. The androgen has an effect in hair follicles either directly or after being converted to dihydrotestosterone by an enzyme called 5alpha-reductase, which is a more potent androgen that have high affinity for attaching androgen receptors(14). Alopecia was not seen in animals given C.spinosa cream(10%) simultaneously with testosterone treatment. In addition to visual observation, quantitative data (follicular density , anagen/telogen ratio , T and DHT serum levels) reveal that C.spinosa inhibits androgenic activity. As a result C.spinosa is regarded to be a viable option for topical use in commercial formulations for androgen-related alopecia and other disorders. Based on our findings, it's possible to conclude that C.spinosa extract works in the same way that finasteride does on hair follicles. C. spinos lowers the androgenic activity of testosterone in serum and hair follicles in general (our findings), but the actual mechanism, whether it is mediated by inhibition of 5 alpha-reductase or an antagonistic action on androgen receptors, is unclear, and more research is needed. Phytochemical screening of C.spinosa plant has revealed the presence of an array of compounds such as alkaloids, flavonoids, steroids, terpenoids and tocopherols (15). Flavonoid compounds such as quercertin and rutin are detected in C. spinosa (16). The presence of flavonoids in C. spinosa can provide antioxidant activity and anti inflammatory activity(17). on the other hand, found that testosterone can cause a shedding of hair through apoptosis of hair follicles rather than the androgen metabolic Pathway (18). As a result, flavonoids may have a role in this plant's ability in hair growth. The present study showed that testosterone induced group and vehicle group significantly increased the testosterone and dihydrotestosterone levels in the serum, ethanolic extract of C.spinosa treatment significantly decrease the testosterone and dihydrotestosterone levels in the serum. Alkaloids compounds that found in C.spinosa plant may be responsible for anti-androgenic effect(19). This preclinical study presents an initial concept for C.spinosa's hair developing ability. This work will be supplemented by additional research into the anti androgenic mechanisms of this herb, in addition to human studies.

Conclusion

Since topical application of C.spinosa promoted hair development and had an anti androgenic impact in testosterone-induced alopecia, it could be considered a promising therapy option for male pattern alopecia and other androgen related disease.

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