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Research Article

EFFECT OF MEDICATED OIL OF MARTYNIA ANNUA LEAVES AND FRUITS ON TESTOSTERONE INDUCED ALOPECIA IN MICE

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ABSTRACT

Objective: The present study aims to evaluate the hair growth promoting activity of leaves and fruits of *Martynia annua* in testosterone induced alopecia in mice.

Methods: Medicated oils of *Martynia annua* leaves and fruits were prepared for topical application as per process mentioned in Ayurveda. Alopecia was induced in albino mice by testosterone (1% w/w) administration subcutaneously for 20 days. Its inhibition by simultaneous administration of medicated oils prepared from leaves and fruits were evaluated using follicular density and anagen/telogen (A/T) ratio, wherein, Finasteride (5alpha-reductase inhibitor) (0.2%) solution served as positive control.

Results: Medicated oil of leaf extract of *Martynia annua* exhibited promising hair growth-promoting activity, as reflected from follicular density and A/T ratio. The treatment was also found successful in bringing greater number of hair follicles in anagenic phase, when compared with control.

Conclusion: Our results concluded that the medicated oil of leaf extract (MOLE) of *Martynia annua* plant shows excellent hair growth activity along with antiandrogenic activity in testosterone induced alopecia model as compared to standard Finasteride.

Keywords: Alopecia, Finasteride, Hair growth, *Martynia annua,* Testosterone.

INTRODUCTION

Hair is one of the vital parts of the body derived from ectoderm of skin, is protective appendages on the body and considered accessory structure of the integument along with sebaceous glands, sweat glands and nails. Hair loss, is dermatological disorder that has been recognized for more than 2000 years and has been estimated to affect between 0.2 % to 2% of world population [1].

Hair suffers several aggressions; and hence there can be some ailments to normal health of hair that causes trouble. The main problems associated with hair such as hair may get thin or fall out, break off or grow slowly, pigmentation problems (fading), dandruff, falling of hair (shedding), scalp inflammation, alopecia etc. Hair loss is generally caused due to genetic tendencies, environmental triggers and exposure to chemicals, medicines, nutritional deficiency, oxidative stress or long illness. Synthetic drugs are more potent and scientifically proved for the treatment of scalp disorders but use of synthetic drugs are associated with many adverse events and generally not advisable for safe and effective treatment [2, 3]. So, drugs of natural origin are necessary to replace the synthetic one and reduce adverse effects associated with them. So we looked into nature's treasure to cope with the problem of hair loss.

Martynia annua Linn. is commonly known in Ayurveda as Kaakanassikaa, belongs to family Martyniaceae. It is traditionally known as Bichchhu [4]. Literature survey revealed that *Martynia annua* Linn. has been used by Santal tribals (India) for hair loss [5]. According to Ayurvedic Pharmacopoeia of India, seed is used in palitya (premature hair graying). Since, no scientific evidence on its hair growth activity is reported, thorough investigation was proposed to be performed over hair growth activity of this crude drug in correlation with traditional claim along with its antioxidant and antiandrogenic activity.

MATERIAL AND METHODS

Sources of chemicals and standard drugs

Testosterone, Finasteride (Sigma–Aldrich Chemie, Steinheim, Germany), Carboxy methyl cellulose Sosium salt (Loba Chemie), Precoated silica gel G 60 F_{254} TLC aluminium plates (20 x 20 cms, 0.2 mm thick) (Merck Ltd. Germany) and AR grade chemicals were used.

Plant material and preparation of extracts

Martynia annua Linn. was collected from local region of Gondia during their flowering season. The plant was botanically identified and authenticated from the Department of Botany, Rashtrasant Tukadoji Maharaj, R.T.M. Nagpur University, Nagpur. A voucher specimen (specimen no. 9271) has been deposited for future reference. The leaves and fruits of *Martynia annua* were plucked, air dried and pulverized to a coarse powder.

Preparation of medicated oil

Medicated oils have principally three components namely, *drava* or *qwatha* (a liquid which may be aqueous decoction of one or more herbs, or juice of herbs or milk), *kalka* (a fine paste of the herbs) and *sneha dravya* (a vegetable oil). Normally, crude Sesame oil (SO) is used as *sneha dravya*, though occasionally, castor oil and coconut oil is also used either in parts or in full. As per AFI, unless otherwise given for any specific Ayurvedic oil recipe, the ratio of the three components are, *kalka* one part, *sneha dravya* (SO) four parts and qwatha should be 16 parts. The general process is to ground the herbs to get coarse powder (# 40), mixed and moistened with just sufficient quantity of water to obtain a fine paste of the herbs (kalka). The raw or powdered herbs (# 10-30), were moistened with water and it is then boiled with 16 times by volume of water to that of herb quantity and continued boiling to reduce the volume to one forth. The decoction was strained using a muslin cloth to obtain the (*qwatha*) a liquid which may be aqueous decoction of herbs. Another component involve sesame oil which was taken in a vessel, heated mildly for some time and mixed with the pasty mass and the aqueous decoction together. This mixture was boiled on mild fire with stirring to avoid *kalka* to adhere to the vessel and boiled till all the water evaporates. Ayurvedic process prescribes to boiling, either till all the water from the decoction evaporates and the moisture in the pasty mass also evaporates. Well-cooked oil should not have any residual moisture (less than 0.1%). The oil was strained while warm through muslin cloth and allowed to cool [6]. From above mentioned procedure, medicated oil of leaves (MOLE) and medicated oil of fruits (MOFE) were prepared from their *qwatha* and *kalka*, individually and stored in amber colored bottle.

Preliminary phytochemical screening

Leaves (MOLE) and fruit (MOFE) of *Martynia annua* were fractionated between polar solvent (ethanol) and non polar solvent

(hexane) and were analyzed for preliminary phytochemical screening for the presence of carbohydrate, alkaloids, glycosides, sterols, flavonoids, phenolics and triterpenoids [7].

Physical and chemical evaluation of medicated oils

The prepared medicated oils were evaluated using standard methods of general characterization, physical and chemical evaluation including Specific gravity, pH, Refractive index, Acid value, Saponification value and Iodine value [8].

HPTLC profile

MOFE, MOLE and plain SO, 1ml each were separately diluted up to 10ml using n-hexane and sample was applied as 4 mm band, under continuous flow of nitrogen, using CAMAG LINOMAT V automatic sample applicator, with the help of 100 µl syringe. Each HPTLC plate precoated, silica gel G 60 F_{254} size 20 x 10 cm was developed to 8 cm in a twin trough glass chamber, saturation time 30 min, scanning mode Absorbance/Reflectance; temperature 20 ± 5°C and separation technique ascending. The optimized solvent system was n-hexane: diethyl-ether: glacial acetic acid [8 : 1.8 : 0.15 (v/v)] and scanned by densitometer (CAMAG TLC scanner-3 with WinCATS software) at 254nm [9].

Experimental animals

Swiss albino mice (25-30g) of either sex were used. The animal studies were approved by the Institutional Animal Ethical Committee (15/2012/CPCSEA) of Department of Pharmaceutical Sciences, Nagpur-440033, India. The animals were fed a standard pellet diet and water ad libitum. They were maintained in a controlled environment and temperature (22 ± 5 ºC with 12-h of light / dark cycle).

Drugs

Sterile testosterone test solution (1%w/w) was prepared as suspension in aqueous carboxyl methyl cellulose solution and was used to induce alopecia in mice. The 2% standard Finasteride solution prepared in vehicle (ethanol/propylene glycol/ water, 8:1:1), served as standard.

Induction of experimental alopecia in mice

Animals were divided into five groups of six animals each and were administered testosterone subcutaneously. Animals of group II, III, IV and V were given topical application of sesame oil (SO), medicated oil of fruit extract (MOFE), medicated oil of leaf extract (MOLE) and standard finasteride solution respectively. Approximately 0.2 ml of solutions was topically applied on back skin once a day for 20 days. After 20 days, mice from each group were selected randomly and sacrificed. Skin biopsy was undertaken from balding site of each group of mice [10, 11].

Histopathological examination

Skin samples were kept in phosphate-buffered formalin for paraffin sectioning. Sections were cut and stained with hematoxyline and eosine. Follicular density (number of hair follicles per mm) and anagen / telogen ratio was calculated with the help of ocular micrometer. Later the microscopic slides of the skin sections were photographed [12].

Statistical analysis

Values are expressed as mean ± SEM. The data were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparision test.

RESULTS

Preliminary phytochemical screening

Leaves (MOLE) and fruits (MOFE) of *Martynia annua* showed presence of alkaloids, tannins, carbohydrates and proteins. Additionally, flavonoids and steroids were also found to be present in medicated oil of leaf extract. Results of Physical and chemical evaluation of medicated oil were within Pharmacopoeial limits (Table 1).

Table1: Results of physico-chemical evaluations

Sr.No.	Parameters	MOFE	MOLE	SO
1	Specific gravity	0.9292	0.9383	0.916
2	pH	7.5	8.3	7.2
3	Refractive index	1.472	1.472	1.476
4	Viscosity (cps)	10.85	11.33	10.82
5	Acid Value	2.13	1.48	1.52
6	Saponification Value	256	198	195

HPTLC profile

Optimized High Performance Thin Layer Chromatogram (HPTLC) of MOLE, MOFE and SO at 254 nm showed the presence of ten components with Rf values (0.06, 0.08, 0.15, 0.19,0.25, 0.30, 0,40, 0.50,0.70, 0.79), eight components with Rf values (0.09, 0.16, 0.25, 0.31, 0.40,0.48, 0.66, 0.74) and three components with Rf values (0.30, 039, 0.47) respectively (Figure 1A, Figure 1B, Figure 1C).

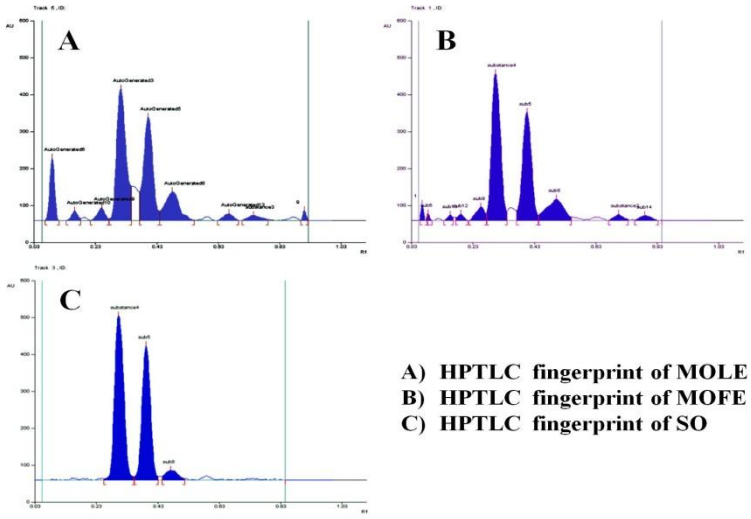

Fig. 1: HPTLC fingerprints of medicated oils and vehicle

Assessment of qualitative hair growth study

Group I and II showed diffuse alopecia as loss of hair from dorsal portion of mice was clearly visible after 20 days of treatment with testosterone (Figure 2A, Figure 2B). In group III, MOFE of *M. annua* was administered along with testosterone the least alopecic condition was observed (Figure 2C). Whereas, in group IV of animal, MOLE of *M. annua* along with testosterone was administered the alopecic condition was not observed (Figure 2D) and group V also showed similar pattern (Figure 2E).

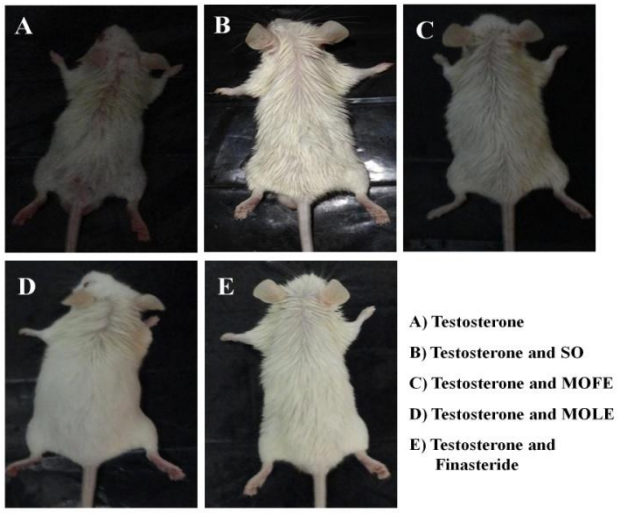

Fig. 2: Qualitative hair growth study of different group of animals

Assessment of anagen/telogen ratio

Anagen/telogen ratio was significantly affected by medicated oils, which was observed as 2.03:1 and 2.42:1 in MOFE-treated group and MOLE-treated group respectively as against 0.95:1 observed for testosterone-treated control and 3.64:1 for finasteride-treated animals (Table 2).

Histopathological study and assessment of follicular density

Follicular density observed in MOFE treated group was 3.15 ± 0.27 and in MOLE treated group was 4.00 ± 0.51(Figure 3C, Figure 3D). Whereas it was 1.74 ± 0.22 in testosterone-treated control, 2.56 ± 0.31 in testosterone SO-treated control and 4.45 ± 0.75 in finasteride-treated standard group (Table 2) (Figure 3A, Figure 3B, Figure 3E).

As evident from above data, activity of MOFE and MOLE was comparable with standard finasteride and control. The predominance of hair follicle in anagenic growth phase indicates reversal of androgen – induced hair loss in MOLE and finasteride-treated group.

Table 2: Follicular density and anagen/telogen ratio of skin sections of different group of animals

Sr. No.	Animal groups	Treatment	Follicular density (no/mm)	Anagen / Telogen ratio
1	I	Testosterone (s.c.)	1.74 ± 0.22	0.95:1
2	II	Testosterone (s.c.) + SO (topical)	2.56 ± 0.31	1.53:1
3	III	Testosterone (s.c.) + MOFE (topical)	3.15 ± 0.27*	2.03:1
4	IV	Testosterone (s.c.) + MOLE (topical)	4.00 ± 0.510**	2.42:1
5	V	Testosterone (s.c.) + Finasteride (topical)	4.45 ± 0.75**	3.64:1

Statistical analysis: Values expressed as Mean±SEM, n=6; * P<0.05, ** P<0.01 and *** P< 0.001 when compared with testosterone group.

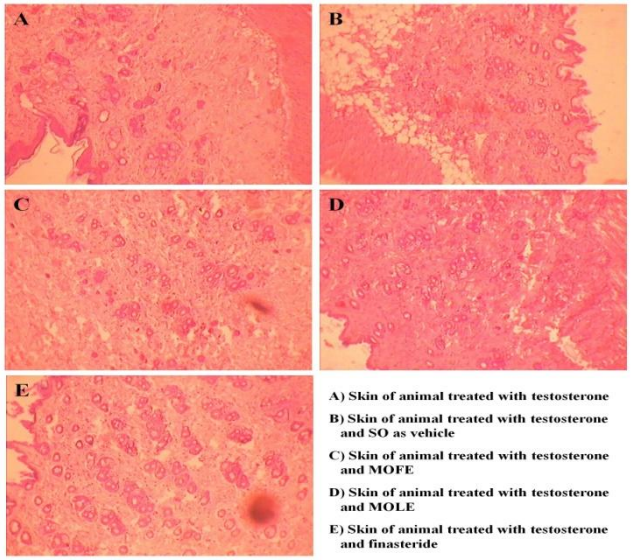

Fig. 3: Histopathological studies of different group of animals

DISCUSSION

The hair growth activity of MOFE and MOLE (2%) was evaluated using testosterone induced alopecia model. From qualitative studies, it was cleared that, amongst the extracts selected for the study, MOLE showed significant hair growth activity. Alopecic condition was not observed in MOLE treated group when compared with the control. From quantitative studies, it was cleared that, the effect of testosterone in miniaturization of hair follicle was blocked by administration of MOLE. The number of hair follicle in anagen phase was considerably increased and fewer follicles in telogen phase were observed. Anagen/telogen ratio was significantly improved and maximum follicular density was found in MOLE treated group of animals when compared with control group. So, besides visual observation and quantitative data (e.g. A/T and follicular density) it also proves inhibition of androgenic activity of medicated oils of *M.annua.* The phytoconstituents such as polyphenols, steroids and saponins present in leaf extracts may be attributed for this activity. Polyphenols have also proved to be effective in many pathological conditions. Also, the *M.annua* plant reported to have significant antioxidant activity [12] which is supported by the fact that potential hair growth property is exhibited by plant possessing antiandrogenic activity and antioxidant activity [13].

CONCLUSION

Our results provide evidence that the medicated oil of leaf extract (MOLE) of *Martynia annua* plant shows excellent hair growth activity along with antiandrogenic and antioxidant activity in testosterone induced alopecia model. Hair growth activity may be attributed to the presence of polyphenol, steroids and saponins in the plant, resulting into increased follicular density and anagen/telogen ratio. However, medicated oil of fruit extract (MOFE) has showed less hair growth activity as compared to MOLE and standard Finasteride. Hence it can be concluded that, medicated oil of leaf extract (MOLE) not only shown remarkable activity but is also found free from potential side effects usually shown by the synthetic drugs. This study provides significant evidence in support of the medicinal and traditional uses of *M.annua* in hair disorders.

Conflict of interest statement

We declare that we have no conflict of interest

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