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Der Pharmacia Lettre, 2016, 8 (19):351-356
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Effect of Alfalfa (*Medicago sativa* L.) Extract on Undesired Hair Growth in Human: A Safe Remedy for Cosmetic Procedures

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ABSTRACT

Hair in humans no longer has any functional use of protecting them from the perils of the environment unlike in other animals. However hair is removed or maintained for many purposes amongst men and women in today's society. Various procedures are used to remove unwanted hair today. However a safe, effective and simple home remedy is what we are looking for in this study. For this purpose the biological properties of Medicago sativa L. (Alfalfa also known as Lucerne), a phytoestrogen plant, is being explored here. Alfalfa extract obtained by plant maceration method and using rotary evaporator for concentration. We use 3 different extract concentration and placebo in this trial. All volunteers were 18-24 years old and with same life style. Data analyzed using SPSS18 software Wilcoxon signed-rank test. Current trials show that the application of alfalfa extract has resulted in human body hair diameter reduction with no side effects. Data analysis proved Correlation between hair growth reduction and alfalfa extract was significant and it was related to its dosage ($P < 0.05$). The actual mechanism behind this effect is not discovered but it should be related to the high concentration of estrogenic components in Alfalfa.

Keywords: *Medicago sativa*; Hair; Phytotherapy.

INTRODUCTION

Hairs appear as elongated keratinized structures developed from invaginations of the epidermal epithelium which is a highly sensitive organ called hair follicle.[1, 2] The hair follicle growth cycle contains three transformation phases called anagen, catagen and telogen which are rapid growth, regression and resting periods with the mean durations of 3 years, 3 weeks and 3 months respectively.[3] Numerous hormones, growth factors and cytokines influence hair growth. They can change follicle size and type but cannot change follicle numbers.[4] Androgen as the most important modulator among hormones and estrogen which is subjected to age and gender play a crucial role in hair growth regulation.[5-7] Androgens are necessary for sebaceous glands development and mediate transformation of the vellus hair into a terminal hair.[8] Other non-steroidal metabolites such as retinoic acid, Vitamin D and thyroid hormones may have effects on hair growth.[9-11] Beside these hormones, growth factors and cytokines such as epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factor beta (TGF-β), nerve growth factor (NGF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and interleukin-1 (IL-1) can also profoundly alter hair growth.[12-16] Phytoestrogen compounds can impact androgen

receptors, suppress Low density lipoprotein (LDL) formation, LDL oxidation and radical chain propagation.[8, 17, 18] Lots of plants particularly legumes produce Phytoestrogen compounds including flavonoids, isoflavonoids and coumestans.[19] Alfalfa (fabaceae family) is one of the legumes which contain coumestrol, apigenin, luteolin, quercetin and medicarpin.[20-22]

Unlike other animals, humans no longer have any functional use of their hair in protecting them from the perils of the environment. However hair is removed or maintained for social, cosmetic and hygiene purposes amongst men and women in today's society and Women's body hair removal is strongly normative.[23] A proper hair removal method for preoperative remedy will cause a saving of approximately 270,000$ per 1000 patient.[24] We are looking for a safe effective and prolonged hair removing method. For this purpose the biological properties of *Medicago sativa* L. (Alfalfa, which also known as Lucerne) is being explored here.[25]

MATERIALS AND METHODS

1.1 Materials

1.1.1 Plant material

Medicago sativa L. leaves (alfalfa) were obtained from Mohammadabad herbal medicine farm of Zabol medical university (31°1'43" N, 61°30'4" E) in Zabol, Sistan and Baluchestan province, Iran.[26] The specimen was confirmed as *Medicago sativa* L. with the help of *Pharmacognosy* division of pharmacy faculty of Zabol medical university. In this experiment about 900g dried plant was obtained from 5kg fresh plant. Crushed plant shoots were macerated in a tank with 80% concentrated ethanol (C_2H_5OH) solvent.[19, 27, 28] After 24 hours the solvent was removed under reduced pressure by rotary evaporator apparatus to obtain required extract in 40°^{C}.[29, 30] In this process 230g pure extract was obtained.

1.1.1 Pharmaceutical material

Eucerine as a basal cream mixed with 1%, 2% and 5% of alfalfa extract.[31]

1.1.1 Exclusion

Any dermal disease such as infectious diseases, eczema, wheals, vesicular and bullous diseases, alopecia, skin and hair malformations and malignancies was considered for exclusion criteria.

1.1.2 Inclusion

All female students above eighteen years old who lived in Zabol University hostel and consumed same food (Hostel food) are eligible according to our protocol.

1.2 Methods

1.2.1 Study protocol

This cross sectional study has been conducted on Zabol University hostel students who had same diet in Zabol, Iran. The study protocol was approved in ethics organized committee of research and technology department in Zabol University of Medical Sciences with the project ethics code of ZBMU.1.REC.1393.5. Written consent was obtained from volunteers before enrollment.

1.2.2 Dermatotoxicity assessment

In order to check alfalfa toxicity, 50% concentrated extract was applied on the bodies of 6 rats. After 4 months use, rat's skin sample studied and no side effect observed.[31]

1.2.1 Clinical assessment

In this Randomized Clinical Trial 60 volunteers aged 18-24 years enrolled in four groups after obtaining a written consent. Each group was having 15 volunteers by simple random sampling. Before using the cream, the length of volunteer hair growth in 4 weeks after epilating was measured. (subject1)

Group no. 1. was treated with a cream containing 1% Alfalfa extract, Group no. 2 was given a cream containing 2% Alfalfa extract , Group no. 3 was given a cream containing 5% Alfalfa extract and Group no. 4 received placebo, which was the vehicle of the cream without Alfalfa extract. The duration of treatment was 12 weeks and the volunteers used the cream twice a day on small area (2cm × 2cm) on their thigh. The decrease in hair growth will be significant only after 3 months therapy.[32] Hence to measure the actual growth period after 3 months from start of

treatment, the area is epilated again. After 1 month from this epilation a measurement is taken of the hair to see the effect of the treatment. (subject2)

1.2.1 Measurement

The hair growth length was noted clinically and hair diameters of subject 1 and subject 2 were measured with a caliper with 0.02 micrometer sensitivity and then compared. All data recorded on data patient data sheet. For understanding about any significant change in hair growth after using alfalfa extract containing cream in comparison with before using it, we used Wilcoxon signed-rank test of statistical package for social sciences software (SPSS Inc., Chicago, IL, USA) version 18.0.

RESULTS

Of the all volunteers who entered the study, 43 volunteers completed the study and 17 were missed in follow-up (4 in group no. 1, 5 in group no. 2 and 6 in group no. 3 and 2 in group no. 4).

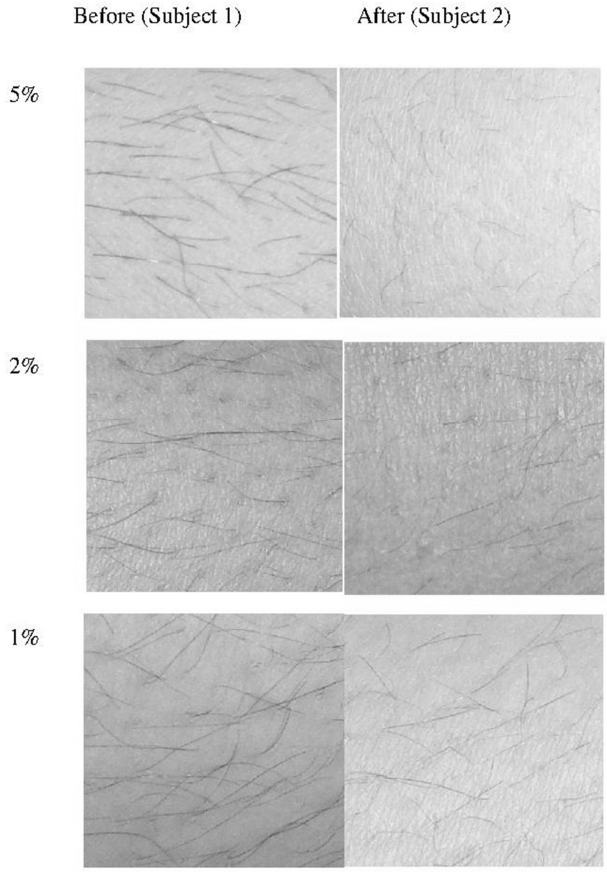

Fig.1 hair growth reduction comparison before and after using the cream containing alfalfa extract

The first sign of clinical efficacy of treatment was reduction in hair growth. The rate of hair growth was only reduced in the three groups treated with 1%, 2% and 5% of Alfalfa cream compared to group which received placebo.

Thigh hair growth diameter was measured in all four groups and the results are shown in table I.

The Standard Deviation (SD) on this data was analyzed. The mean value of reduction of hair diameter was 16% (SD = 0.78) for volunteers who received the creams containing 1% alfalfa alcoholic extract, 25% (SD = 0.93) for volunteers who received the creams containing 2% alfalfa alcoholic extract, 36% (SD = 0.81) for volunteers who

received the creams containing 5% alfalfa alcoholic extract and 3.7% (SD = 0.76) for volunteers who received the creams containing 0% (placebo).

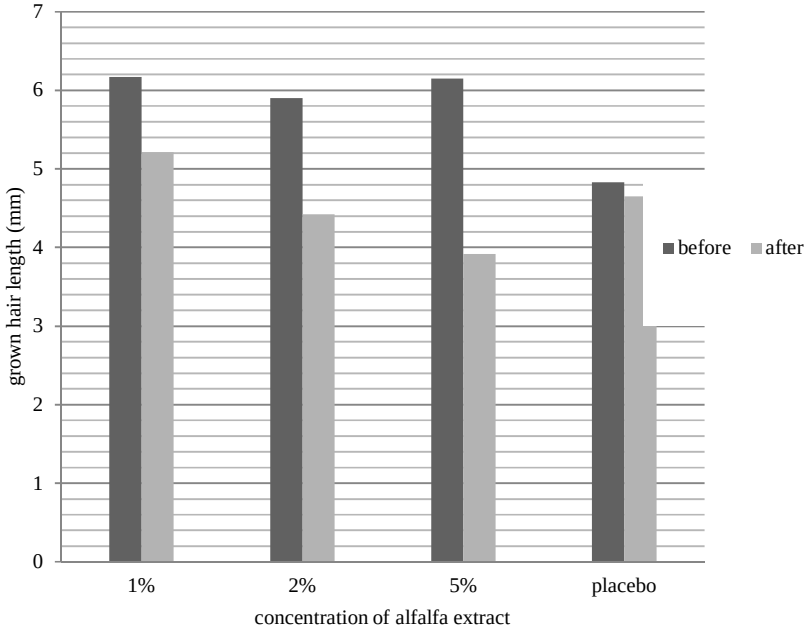

Fig. 2 comparison scheme of hair reduction diameters

TABLE I HAIR REDUCTION DIAMETERS

Dose	Duration (weeks)	IAL (mm)| SD	FAL (mm)| SD	R%
1%	12	6.17mm SD=1.20	5.21mm SD= 0.78	16%
2%	12	5.90mm SD=1.40	4.42mm SD=0.93	25%
5%	12	6.15mm SD=0.94	3.92mm SD=0.81	36%
placebo	12	4.83mm SD=0.69	4.65mm SD= 0.76	3.7%

IAD = Initial Average Length, FAD = Final Average Length, SD = Standard Deviation, R = Reduction.

Data analysis with using SPSS software proved Correlation between hair growth reduction and alfalfa extract dosage was significant. (P value<0.05) The Wilcoxon signed-rank test showed that there is a significant change after using the extract containing cream in all dosages. (P value=0.047 for 1% dosage, P value= 0.041 for 2% dosage and P value=0.036 for 5% dosage)

These results show that there is more significant change after using 5% alfalfa extract containing cream and the best clinical efficacy and most volunteer satisfaction were achieved with the cream containing 5% Alfalfa extract. (Fig 1, 2)

CONCLUSION

Androgens are male hormones produced in the bodies of both men and women. Body hair growth is related to androgen level as it can stimulate follicle and sebaceous cell function. Estrogen components with suppressing of LH level (luteinizing hormone) reduce ovarian androgen production.[17] Alfalfa is known as one of main legumes with high concentration of estrogens in the world. It is known as female plant and used in women diet to this date in Iran. The expected effects of this plant in Iran traditional medicine are sexual cycle regulation, body hair decrease, body fitness, genital system regulation, breast growth controller, breast feeding promoter, etc. Alfalfa's phytoestrogenic activities were studied in various experiments. Therefore we have tried the effect of this plant on human hair growth. Our results showed topical usage of alfalfa extract can be used as a safe remedy for undesired hair reduction.

There are several studies on phytoestrogens. Javidnia and colleagues showed that fennel extract had inhibiting effect on hair growth of hirsute patients.[33] In another study Seiberg showed using soy bean extract reduced mice hair growth compared with Isoflavones.[25] Inaoka and his colleges in a widespread program evaluated the effect of eighty types of herbs on hair growth. They have demonstrated that some herbs decreased and some promoted hair growth. [34] Current trials show that the application of alfalfa extract has resulted in human body hair diameter reduction with no side effect. The actual mechanism behind this effect is not discovered but it should be related to the high concentration of estrogenic components in Alfalfa. Therefore we propose further trials for dosage standardization and hirsutism treatment.[35]

Acknowledgment
The authors acknowledge Zabol University of Medical Sciences research and health assistances for their cooperation during the study.

Ethical statement: Nevertheless Alfalfa is used as a vegetable in Iran, we examined Its effect on rat skin with higher dosage for any allergy or adverse effect. No adverse effects were found in pathologic observations.

Patient documents remained secure in this study according to the Zabol University of Medical Sciences ethical laws.

Conflict of interest: The authors declare that they have no conflict of interest.

REFERENCES

[1] C.N. Lawson, J. Hollinger, S. Sethi, I. Rodney, R. Sarkar, N. Dlova, V.D. Callender, *International Journal of Women's Dermatology*, 1 (**2015**) 59-75.
[2] J.C. Luiz Carlos Uchôa, and Alexander N. Contopoulos, Junqueira's Basic Histology: Text & Atlas, Mcgraw-hill medical New York, **2005**.
[3] A. Slominski, R. Paus, P. Plonka, A. Chakraborty, M. Maurer, D. Pruski, S. Lukiewicz, *The Journal of investigative dermatology*, 102 (**1994**) 862-869.
[4] S. Müller-Röver, B. Handjiski, C. van der Veen, S. Eichmüller, K. Foitzik, I.A. McKay, K.S. Stenn, R. Paus, Journal of Investigative Dermatology, 117 (**2001**) 3-15.
[5] G. Wehner, H.U. Schweikert, *The Journal of clinical endocrinology and metabolism*, 99 (**2014**) 1393-1399.
[6] R. Choudhry, M. Hodgins, T. Van der Kwast, A. Brinkmann, W. Boersma, *Journal of Endocrinology*, 133 (**1992**) 467-NP.
[7] H.-S. Oh, R.C. Smart, *Proceedings of the National Academy of Sciences*, 93 (**1996**) 12525-12530.
[8] F.A. Longo DL, Kasper DL, Hauser SL, Jameson JL, Loscalzo J. Harrison's Principles of Internal Medicine 18 ed. united states of America McGraw Hill Professional; **2012**. 3610 p.
[9] K. Foitzik, T. Spexard, M. Nakamura, U. Halsner, R. Paus, *Journal of investigative dermatology*, 124 (**2005**) 1119-1126.
[10] Z. Xie, L. Komuves, Q.-C. Yu, H. Elalieh, D.C. Ng, C. Leary, S. Chang, D. Crumrine, T. Yoshizawa, S. Kato, *Journal of investigative dermatology*, 118 (**2002**) 11-16.
[11] A. Messenger, *British Journal of Dermatology*, 142 (**2000**) 633-634.
[12] T.A. Rosenquist, G.R. Martin, *Developmental dynamics*, 205 (**1996**) 379-386.

[13] E.M.J. Peters, B. Handjiski, A. Kuhlmei, E. Hagen, H. Bielas, A. Braun, B.F. Klapp, R. Paus, P.C. Arck, The *American journal of pathology*, 165 (**2004**) 259-271.
[14] L. Karlsson, C. Bondjers, C. Betsholtz, *Development*, 126 (**1999**) 2611-2621.
[15] K. Yano, L.F. Brown, M. Detmar, *The Journal of clinical investigation*, 107 (**2001**) 409-417.
[16] C. Harmon, T. Nevins, *Lymphokine and cytokine research*, 12 (**1993**) 197-203.
[17] M.P. Philpott, T. Kealey, *Journal of investigative dermatology*, 102 (**1994**) 186-191.
[18] R. Hoffmann, W. Eicheler, A. Huth, E. Wenzel, R. Happle, *Archives of dermatological research*, 288 (**1996**) 153-156.
[19] J. Hwang, H.N. Hodis, A. Sevanian, *Journal of agricultural and food chemistry*, 49 (**2001**) 308-314.
[20] Y.-H. Hong, S.-c. Wang, C. Hsu, B.-F. Lin, Y.-H. Kuo, C.-j. Huang, *Journal of agricultural and food chemistry*, 59 (**2010**) 131-137.
[21] S.M. Tsai, D.A. Phillips, *Applied and Environmental Microbiology*, 57 (**1991**) 1485-1488.
[22] A.M. Patten, M. Jourdes, E.E. Brown, M.-P. Laborie, L.B. Davin, N.G. Lewis, *American journal of botany*, 94 (**2007**) 912-925.
[23] P. Seguin, W. Zheng, A. Souleimanov, *Journal of Agronomy and Crop Science*, 190 (**2004**) 211-217.
[24] D. Smith, G. Paulsen, C. Raguse, *Plant Physiology*, 39 (**1964**) 960.
[25] M. Seiberg, Soybean-Biochemistry, Chemistry and Physiology, Ng, TB (Ed.). InTech Publishers, New York, USA, (**2011**) 119-136.
[26] J.W. Alexander, J.E. Fischer, M. Boyajian, J. Palmquist, M.J. Morris, *Archives of Surgery*, 118 (**1983**) 347-352.
[27] T. Cholastova, D. Knotova, *World Academy of Science, Engineering and Technology*, 69 (**2012**) 856-862.
[28] J. Sharifi-Rad, S.M. Hoseini-Alfatemi, M. Sharifi-Rad, M. Sharifi-Rad, M. Iriti, M. Sharifi-Rad, R. Sharifi-Rad, S. Raeisi, *Molecules*, 20 (**2015**) 7034-7047.
[29] P.M.A.s.p.o.l.J.o.B.C. **1951**;188(2):471-6.
[30] R. McCready, J. Guggolz, V. Silviera, H. Owens, *Analytical chemistry*, 22 (**1950**) 1156-1158.
[31] S. Nasri, G. Amin, A. Azimi, World Academy of Science, Engineering and Technology, *International Journal of Medical, Health, Biomedical, Bioengineering and Pharmaceutical Engineering*, 8 (**2014**) 186-189.
[32] A. Mustafa, C. Turner, *Analytica chimica acta*, 703 (**2011**) 8-18.
[33] K. Javidnia, L. Dastgheib, S. Mohammadi Samani, A. Nasiri, *Phytomedicine*, 10 (**2003**) 455-458.
[34] Y. Inaoka, A. Shakuya, H. Fukazawa, H. Ishida, H. Nukaya, K. Tsuji, H. Kuroda, M. Okada, M. Fukushima, T. Kosuge, *Chemical & pharmaceutical bulletin*, 42 (**1994**) 530-533.
[35] A. Nostro, M. Germano, V. D'angelo, A. Marino, M. Cannatelli, *Letters in applied microbiology*, 30 (**2000**) 379-384.