

EFFECT OF GELATIN-CYSTINE AND SERENOA REPENS EXTRACT ON FREE RADICALS LEVEL AND HAIR GROWTH

P. Morganti*, G. Fabrizi**, B. James***, C. Bruno****

*President/Director, R. & D - Mavi Sud S.r.l., Aprilia (LT), Italy

Head Department of Cosmetic Dermatology, Accademia di Storia dell'Arte Sanitaria, Rome, Italy

** Department of Paediatric Dermatology, Catholic University "Sacro Cuore", Rome, Italy

*** Program Director ISCD; Consultant AMERx - 157 Beacon Street #2 Boston, MA 02116 - USA

**** Department of Anatomy and Natural Physiology, University of Urbino, Italy

Received: June 29, 1998

Presented at "Singapore Clinical Dermatology 2000" - Singapore, 18 - 20 June, 1998

Key words: Hair growth, androgenetic alopecia, seborrhea, serenoa repens, cystine, gelatin-cystine, cosmeceutical, nutraceutical.

Synopsis

The influence of gelatin-cystine and serenoa repens on hair growth was studied through a long period application (50 weeks) of a new cosmeceutical lotion. On 48 volunteers (24 women and 24 men) aged between 21 and 38 years, affected by androgenetic alopecia (type III and IV according to Hamilton). 12 subjects also took a diet supplement (4 pills per day) based on gelatin-cystine. The solution and the diet supplement (placebo and active) were assigned in a randomized double-blind manner. Hair mass and the mean hair number were controlled according to Price et al. Exclusion criteria included use of topical or oral drug or diet supplement within the previous six months.

The obtained results showed an increase of hair mass from 20 to 30% ($p < 0.005$) together with a contemporary increase of hair number (from 17 to 27%) ($p < 0.005$) compared to the placebo for subjects using the lotion only.

With the diet supplement a further increase of 50% ($p < 0.005$) in hair growth and a significant decrease of blood ROS (Reactive Oxygen Species) were obtained.

Riassunto

L'influenza della gelatina-cistina e della serenoa repens sulla crescita dei capelli è stata studiata per un lungo periodo (50 settimane) applicando una nuova lozione cosmeceutica su 48 volontari (24 donne e 24 uomini) in età compresa tra i 21 e 38 anni, affetti da alopecia androgenetica (III tipo e IV secondo Hamilton).

12 soggetti sono stati sottoposti ad assunzione di un dietetico (4 pillole al giorno) a base di gelatina-cistina.

La lozione ed il dietetico (placebo e principi attivi) sono stati assegnati in modo casuale e a doppio cieco. Il peso totale ed il numero medio dei capelli è stato controllato secondo Prince et al. Il criterio di esclusione

includeva l'assunzione di sostanze medicinali per via topica, orale o di diete speciali assunti nei precedenti sei mesi.

I risultati ottenuti con la lozione attiva rispetto al placebo mostrano un incremento del peso totale dei capelli dal 20 al 30 % ($p < 0.005$) insieme ad un contemporaneo incremento del numero di capelli dal 17 al 27% ($p < 0.005$) rispetto al placebo.

Con la contemporanea assunzione del dietetico è stato ottenuto un ulteriore incremento del 50% ($p < 0.005$) nella crescita dei capelli ed una diminuzione statisticamente significativa dei ROS nel sangue.

INTRODUCTION

According to our research (1-4) gelatin-cystine by oral route seems to induce hair growth. Moreover liposteroidal and alcoholic extracts of *Serenoa repens* also seem to assess effect on retarding the hair loss process in androgenetic alopecia inhibiting the 5- α -reductase (type II isozyme) activity and therefore the binding of DHT (Dehydrotestosterone) to androgen receptors (5-10). Moreover it appears that tissue sensitivity to circulating androgens and the role of 5- α -reductase activity within the hair follicle is of primary importance in the expression of androgenetic alopecia hair loss (11). The *serenoa repens* by topical or oral route seems to be comparable with finasteride without exerting the adverse side effects of a drug (12-14).

AIM

The aim of this work was two-fold:

- to control the efficacy of both the oral gelatin-cystine and the cosmeceutical lotion based on l-cystine and *serenoa repens* extracts on hair growth promotion and retarding of hair loss ;
- to quantify the radical oxygen species (ROS) before, during and after the diet supplementation.

There is increasing evidence that ROS may be involved in a variety of skin disorders and excessive production of these species occurs in certain disease states or as a result of toxic insult by selected foreign compounds drugs (xenobiotics) (15,16).

MATERIALS AND METHODS

Materials

LOTION A (Active)

Aqua (water), alcohol denat, Hydrolyzed soy protein, ethoxydiglycol, disodium cystinyl disuccinate, PPG-buteth 26, PEG-40 hydrogenated

castor oil, panthenol, ginkgo biloba (ginkgo biloba extract), carboxymethyl betaglukan, serenoa repens (saw palmetto extract), azelaic acid, piroctone olamine, ethyl nicotinate.

LOTION B (Control)

Aqua (water), alcohol denat, Hydrolyzed soy protein, ethoxydiglycol, PPG-buteth 26, PEG-40 hydrogenated castor oil, panthenol, ginkgo biloba (ginkgo biloba extract), carboxymethyl betaglukan, azelaic acid, piroctone olamine, ethyl nicotinate.

DIET SUPPLEMENT

Diet Supplement (Active)

Soy oil and gelatin, l-cistin, l-methionin, Cu, Zn.

Diet Supplement (Placebo)

Soy oil and starch

APPARATUS

- 3C System® (Rome, Italy)
- ROS-meter System® (Rome, Italy)

Methods

36 volunteers (24 women and 24 men) aged between 21 and 38 years, affected by androgenetic alopecia (type III and IV according to the Hamilton scale), were enrolled in the study for 50 weeks.

Concurrently another group of 24 subjects took also a diet supplement (4 pills a day) based on gelatin-cystine or placebo and their hair growth was compared with the other treated groups. The cosmeceutical solutions and the diet supplements were assigned in a randomized double-blind manner as follows:

12 (6 women and 6 men) received active lotion A (group 1), 12 placebo lotion B (group 2), 12 diet supplement active C (group 3), 12 supplement placebo D (group 4) and 12 lotion A and diet supplement C (group 5).

The subjects were instructed to apply the lotion two times a day (8 a.m. and 8 p.m.). They received also a mild shampoo (17) to be used during

the whole study and excluded use of topical or oral drug and diet supplement within the previous six months.

• Area Treated

A pre-selected frontal/parietal scalp area 1 cm² was hand clipped on day 0 and at 10 weeks intervals there after for a total of 50 weeks. Blood samples (0.2 ml) were also taken from the finger at 8 a.m. (day 0) and weekly for 12 weeks to control ROS.

• Measurement Method

According to Price and Menefee methodology (18, 19), hair samples were degreased in Freon TF, dried, counted on a grid and then weighed in an analytical balance at 22°C and RH 50%.

• Statistics

Paired student t-test and analysis of variance (ANO-DIA) for changes in time were used to detect significant effects of treatments.

• ROS Determination

ROS were determined by the new D-ROMS test which is based on the capability of transition metals to catalize the formation of free radicals in presence of peroxides (20). The free radicals produced, whose quantity is directly proportional to the quantity of peroxides present in the plasma, are trapped chemically by molecules of a phenolic derivative, changed into chromophores and evaluated photo-

metrically by ROS-meter System at 505 nm. By this methodology the normal value in blood (serum or plasma or whole blood) are within the range of 250 and 300 U.CARR conventional units. The value of 1 U.CARR correspond to a concentration of 0.08 mg % of hydrogen peroxide. Subjects with value >320 U.CARR can be considered affected by oxidative stress according to the table I.

• Safety Control

The following safety parameters were assessed at each control every each month for all the period of the study. A general medical examination, blood pressure, pulse, and local tolerance such as erythema, stinging/burning and itching. For three times at beginning of the study (time 0) and at the 30th and the 50th week was controlled: the complete blood counts and urine analysis for comparing any changes from baseline.

Table I

U. CARR VALUES

U. CARR		Dosable ROS
from	300 to 320	border - line
from	320 to 340	low
from	400 to 500	high
>	500	very high

RESULT AND CONTROL

The tolerance both of the lotion and the diet complement was excellent for all subjects. No side effect was recorded.

As it is shown in Fig. 1 the sole topical treatment performed with the lotion caused an increase in hair number of 17% ($p < 0.005$) starting from week 10. This increase continued steadily throughout the study, and reached 27% ($p < 0.005$) at week 50. If we compare the increased hair number with their mass (Fig. 2) we also note a gradual increase in hair weight and eventually in hair caliber until a value of around 20% ($p < 0.005$) is reached at week 10. At week 50 the increase recorded is around 30%. This means an increase in hair growth during the anagen phase, and thus a turnabout of hair miniaturization which is typical of androgenetic alopecia.

This result is undoubtedly due to the activity performed by the extract of serenoa repens, which, as is known, opposes the transformation

of testosterone in dehydrotestosterone. Also, it is bound to the contemporary backing presence of l-cystine at level of hair keratogenetic area. These two activities performed at two different levels are possible thanks to the vehicle that enhances transcutaneous penetration of the active ingredients, as it was proved by another study (21).

The dietetic gelatine-cystine product (Fig. 1) acts in a similar way, causing a natural increase in hair number of 18% ($p < 0.005$) after week 10 and of 29% ($p < 0.005$) at week 50. This confirms our previous data (1-4). Indeed in this study it is also proved that gelatine-cystine causes an increase in hair mass of 24% ($p < 0.005$) after 10 weeks and of about 40% ($p < 0.005$) at week 50 (Fig. 2).

It is also interesting to note that the contemporary use of lotion and dietetic supplement cause a further increase of about 50% ($p < 0.005$), if compared with the sole use of lotion or dietetic. Such increase is evident both in number and hair mass starting from the first week of treat-

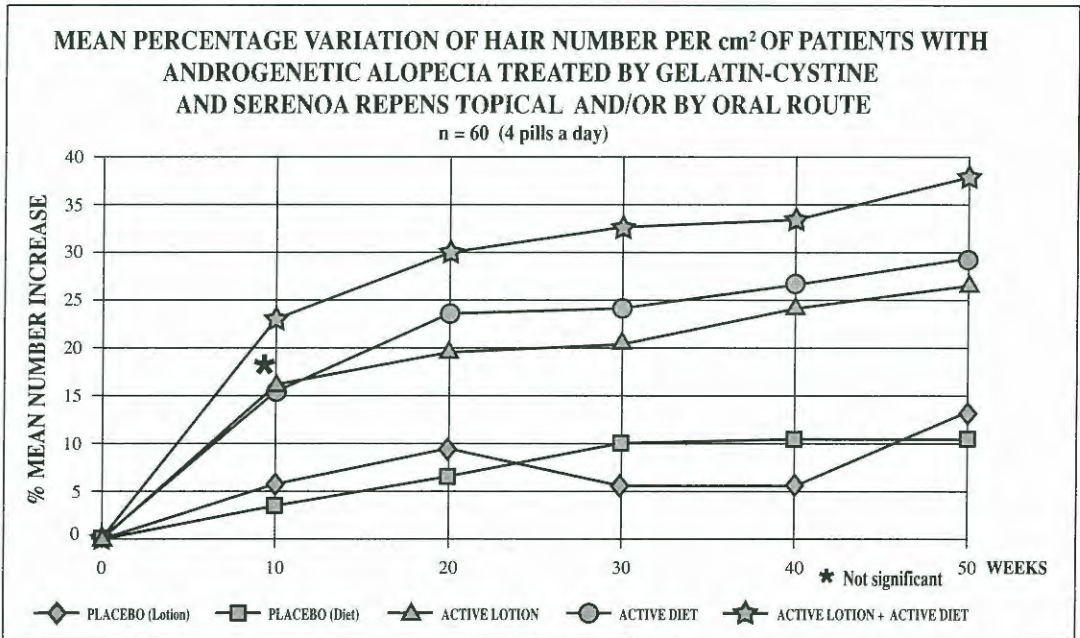


Fig. 1. All p values are highly significant ($p < 0.005$) as baseline values and as to groups.

MEAN PERCENTAGE VARIATION OF TOTAL HAIR MASS PER cm² OF PATIENTS WITH ANDROGENETIC ALOPECIA TREATED BY GELATIN-CYSTINE AND SERENOA REPENS TOPICAL AND/OR BY ORAL ROUTE

n = 60 (4 pills a day)

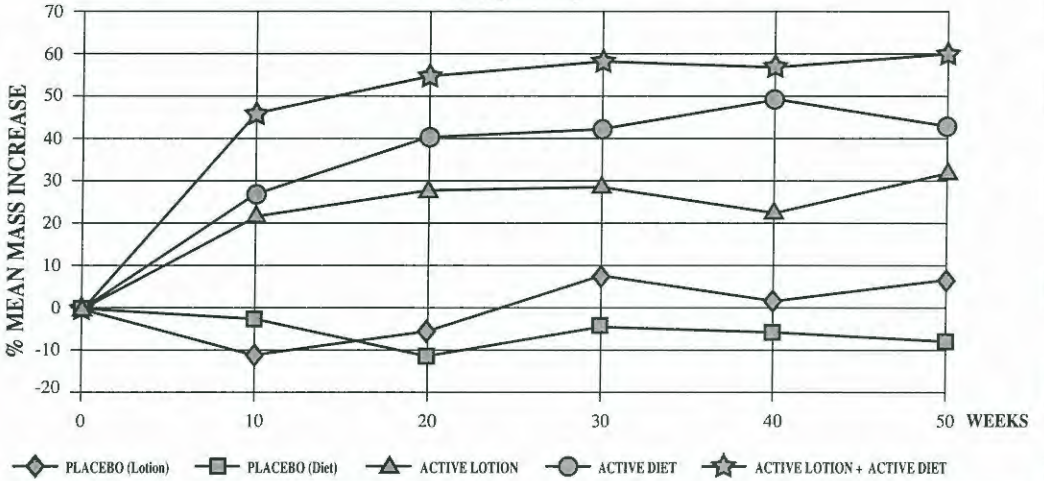


Fig. 2. All p values are highly significant ($p < 0.005$) as baseline values and as to groups.

ROS RECOVERY IN PATIENTS (MALE AND FEMALE) WITH ANDROGENETIC ALOPECIA TREATED BY GELATIN-CYSTINE AND SERENOA REPENS TOPICAL AND/OR BY ORAL ROUTE

n = 60 (4 pills a day)

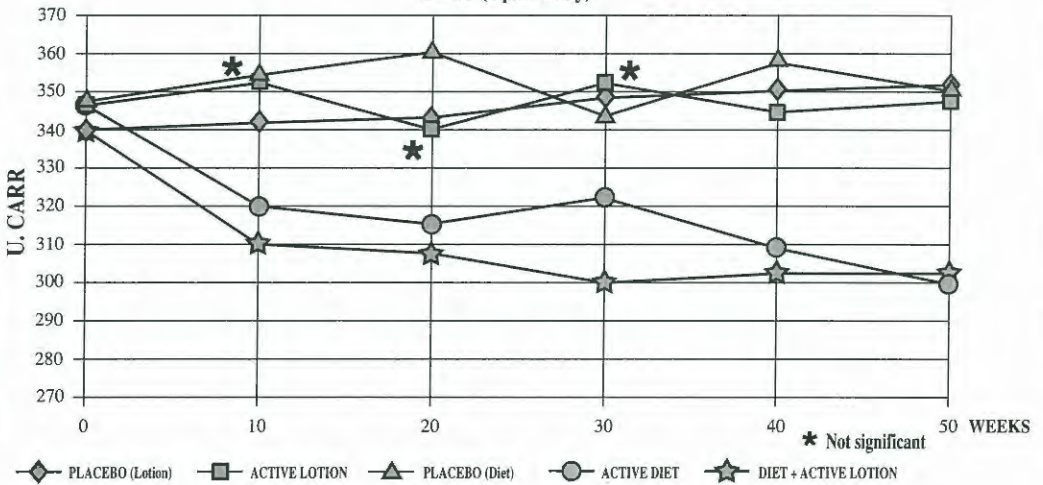


Fig. 3. All p values are highly significant ($p < 0.005$) as baseline values and as to groups.

ment, and increases proportionally until it reaches its peak at week 50 (Fig. 1-2).

Concerning ROS control it should be considered that all groups presented at the beginning of the study values (340 ± 15) that prove, according to Tab. 1, a slight oxidative stress.

As it is shown in Fig. 3 this slight stress stays almost unchanged in groups treated with the lotion or placebo both topical and oral, whilst it goes back to normal values in groups treated with the sole dietetic or with dietetic and lotion. Thus we can deduce that the only activity performed on ROS is carried out by gelatin-cystine by oral route. These first laboratory data prove the possibility of treating subjects suffering from androgenetic alopecia not only with drugs but also with cosmeceuticals and nutraceuticals appropriately formulated and capable of affecting hair regrowth as well.

Moreover, these data seem to confirm the activity performed at pilosebaceous bulb level both by I-cystine and by antiandrogens contained in *Serenoa Repens*.

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Author Address:

Pierfrancesco Morganti, Ph. D.

Via Innocenzo XI, 41

00165 Rome Italy

Tel: +39.6.92.86.261 - Fax: +39.6.92.81.523

E-mail: mavi@colosseum.it