

Clinical Study

ACNISTAT

Acnistat Anti-seborrheic Shampoo

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Research, Production, Pharmaceuticals

Acnistat Anti-seborrheic Shampoo – Clinical Study

Theoretical considerations concerning seborrhea physiopathology

The seborrheic syndrome has at its base a dysfunction of the pilo-sebaceum produced by the intervention of certain factors which determine the increase of sebaceous secretion. This syndrome is determined by hormonal imbalances, particularly the morphology of the pilo-sebaceum and secondary factors of increased sebaceous secretion, namely: the development of the microbial saprophyte flora (*malassezi* / *pityrosporum ovale*) and secondary lipolysis.

a) Hormonal Imbalances: During puberty, there is an increase in the synthesis of androgens, due to the activation of the testicles, of the ovaries, and the suprarenal by the pituitary (*hypophysial* / *hypophyseal*) and the hypothalamus, following certain preexistent genetical factors. The sebaceous glands, being a veritable receptor of androgenic hormones react by producing sebum. The importance of the endocrine factor has been suggested by a series of clinical aspects such as: the absence of seborrhea before puberty, its absence in those castrated or suffering from hypogonadism, and the association of seborrhea with a series of virilization syndromes in women. Androgenic hyper-secretion determines a rise of sebaceous secretion and a hyperkeratosis of the follicular ostium which constitutes the principal factor in the apparition of the seborrheic syndrome.

b) Functional particularities and morphology of the pilo-sebaceous follicle. It is customary to consider that at the onset of seborrhea there exists a morbid hereditary predisposition; the dysfunction of the pilo-sebaceous follicle implies as much the formation of localized androgens (androgenic hormones) at the cellular level, as well as an increased receptivity towards these hormones, fact which explains the appearance of the seborrheic syndrome during normal levels of circulating androgens. The hyper-sensitivity of follicular cells explains why the sebaceous glands are well developed, having a secretory capacity over 20 times larger than in normal conditions. The formation of androgenic hormones by the pilo-sebaceous follicle is demonstrated by the raised enzymatic activity in 3-beta-HSD (3-beta-hydroxysteroid dehydrogenase), which intervenes in the oxido-reduction of androgenic hormones, reactions which transform 4-androstenedione produced by suprarenal into DHT (Dihydrotestosterone), which is considered to be 20 times more active than testosterone. Furthermore, in the pilo-sebaceous follicle, the enzyme 5-Alpha reductases, enzyme which transforms free testosterone into its active metabolic DHT, has been found. The enzyme couples with a cytoplasmic protein which transports it to the nucleus, where complex biosynthesis processes of the sebaceous glands intervene.

c) Secondary factors of increased sebaceous secretion. Hyper-secretion of androgens and hyper-sensitivity of the pilo-sebaceous follicle affect the exaggerated development of the sebaceous glands and their hyper-atrophy, followed by the blockage of sebum flow in the follicle. Sebum retention favors the appearance of certain anaerobic conditions which ease the development of the microbial flora

(*Propionibacterium acnes* & *Staphylococcus epidermidis*). These germs synthesize lipolytic enzymes which produce sebum lysis by the release of free fatty acids (C8-C14) which are unusually irritating and help initialize the inflammatory process in seborrhea and acne. These free fatty acids, in excess, produce skin irritation and local inflammatory reactions which increase the secretion of the sebaceous glands. Furthermore, the distension of the ducts and the follicles bring about the alteration and destruction of their walls and the leaking of follicle content into the dermis, which has as a consequence an inflammation similar to that of a foreign body reaction; the keratin and the sebum behaving accordingly. On another note, the germs can they themselves generate inflammatory reactions through the production of inflammatory enzymes (protease & amylase) which activate the macrophages and exert a chemotaxis for the neutrophilic leucocytes. The increased appearance of neutrophils around the affected follicle is followed by the release of collagens and elastase thus resulting in an increase in inflammatory reactions. The healing of this inflammation is cicatrization with the destruction of the pilo-sebaceous follicle, a fact which explains why once the onset of alopecia appears, it remains definitively. In the aggravation of seborrhea, there are a series of factors that intervene such as: infection by pyogenic germs or Gram negative germs, applying fatty substances, cortisone products, detergents, iodine based medicines, bromine, barbiturates, spicy foods.

Physiopathologic treatment of seborrhea

The physiopathologic treatment of seborrhea implies:

1. Intercepting the androgenic hormonal actions and their metabolites at the androgens receptor level of the pilo-sebaceous follicle
2. Treatment of subclinical local inflammatory processes
3. Stimulating local vascularization and regeneration of the polisebaceous follicle

Acnistat – Anti-seborrheic shampoo, addresses all of the above concerns.

Acnistat – Anti-Seborrheic shampoo, contains a series of active substances with local androgenic actions which selectively block androgen receptors at the poli-sebaceous follicle level. This fact brings about a decrease in activity of the sebaceous glands and the clinical consequences are among the best. In this sense, based on a series of clinical results and observations, we used a series of plant products which contain chemical substances similar in structure to androgenic hormones, namely phytohormones. These substances, although in minute quantity, selectively block the hormonal receptors at the sebaceous gland level in such a way as to incapacitate the activation of the sebaceous glands. Maximum effect is achieved locally with secondary systemic effects absent. Acnistat – Anti-Seborrheic shampoo impedes androgenic hormone actions and its metabolites from acting on the sebaceous

glands. The clinical effect is of maximum importance and efficiency: after a month or a month and a half of applying Acnistat Shampoo, results are among the best: absence of dandruff and total stopping of hair loss. Other components of the shampoo (Dermal 115 and Zinc, as well as the Acnistat lotion) address the other aspects of seborrhea. Since sebaceous glands and sudoriferous glands are stopped only through local action of the shampoo, it is understood that in the absence of administering this shampoo, after a while, the syndromes associated with seborrhea begin to reappear.

Study objectives

In conducting this experiment, many aspects were followed such as:

- I. Level of sebum: quantitative determination, before and after the administration of the Acnistat - Shampoo**
- II. The chemical composition of the sebum before and after administration**
- III. The study of the microbial flora of the scalp before and after administration**
- IV. Particular aspects - pruritus, dry seborrhea, wet seborrhea, hair loss**

I. Level of sebum: quantitative determination, before and after the administration of the Acnistat - Shampoo. Materials and Methods

The study followed the measurement of the normal level of sebum (casual level), which represents the level of saturation in conditions where the tested area is not protected against inevitable touching, wiping or contact with bedding or clothing. It is important because it offers a relation with the daily living conditions.

The collection of sebum was done by following a simple method of a sterile cotton swab, degreased with alcohol and dried in a drying oven, and attached to a swab holder. The cotton swabs was kept in clean glass container, degreased and hermetically sealed. The handling of the cotton swabs was performed only with tongs to prevent cross contamination with the hand and to reduce error factors.

For the experimental group, 10 people were selected with seborrheic secretions and hair loss, of male and female sexes in ages from 20 - 26 years. The collection sites were on the scalp, at the vertex level, subjectively chosen on a 1x2cm surface. Collection was done by cotton swabs on this surface.

A first collection was done before the use of the prepared substance. Washing with this preparation was done with energetic massaging of the scalp and was left on the scalp for approximately 5 minutes. Collecting samples was performed on the first, second, and the fourth days following the use of the shampoo, as well as a collection of samples during the third washing.

In order to exclude that the seborrhea and hair loss were caused by other pre-existing conditions, all subjects were clinically and para-clinically examined. The lab research targeted the following:

- Hormonal dosing specifically of urinary 17-cetosteroid in urine every 24 hours in order to exclude a supra-reno-genital syndrome.
- Determining cholesterol and lipids levels in order to rule out possible subclinical hypo-thyroid condition
- Hemo-leuco-gram with complete morphology in order to exclude blood affections: anemia, leukemia, etc.

Results from lab probes are included in the following tables:

	Case	Age	Sex	17 CST mg/urin in 24 hrs	Cholesterol (mg %)	Total Lipids (mg %)	Hemo-leuco-gram				
							Hematics	Reticulocyt e	Hb	Leukocyte	Thromboc yte
1.	P.I	24	fem.	10	2	550	4,5	1	1 2	6000/mm ³ PMN=67% L=26% E=1% M=6%	200.000 mm ³
2.	B.M	26	fem.	11	1,8	600	4,3	1,2	1 1, 5	5000/mm ³ PMN=70% L=24% E=2% M=4%	180.000 mm ³
3.	M.C	24	fem	10,5	2,1	560	4,8	1	1 3	7500/ mm ³ PMN=65% L=27% E=2% M=6%	210.000 mm ³
4.	C.R	24	male	10	2,25	750	4,7	1,1	1 2, 5	6000/mm ³ PMN=64% L=27% E=2% M=9%	270.000 mm ³
5.	L.C	25	fem.	9	2	580	4,4	1,2	1 2	5500/ mm ³ PMN=62% L=29% E=1% M=8%	220.000 mm ³
6.	V.D	24	fem	11	1,9	720	4,2	1	1 1	6000/ mm ³ PMN=68% L=26% E=2% M=4%	300.000 mm ³

7.	P.G	20	fem	10,5	1,95	620	4,3	1,1	1 1, 5	7000/ mm ³ PMN=66% L=26% E=15 M=7%	300.000 mm ³
8.	L.G	24	fem	11	2,3	590	4,1	1,2	1 1	7500/ mm ³ PMN=66% L=26% E=2% M=7%	300.000 mm ³
9	D.C	26	fem	11,5	2,3	780	4,5	1,2	1 2	7400/ mm ³ PMN=64% L=27% E=2% M=7%	310.000 mm ³
10	P.C	26	fem	10,5	1,95	570	4,6	1,1	1 3	6500/ mm ³ PMN=68% L=22% E=15 M=9%	300.000 mm ³

Values are well within normal limits.

Results

Determining the level of sebum was obtained by weighing the tampons used for collecting the sebum before and after treatment and the differences in weight.

The quantity of the sebum in grams is shown in the following table.

Level of sebum before and after the use of the shampoo in the study group.

Nr crt	Case	Age	Sex	Before Treatment (g)	First Day Following Treatment (g)	Second Day Following Treatment (g)	Fourth Day Following Treatment (g)	In time after a number of washings
1.	I.D	23	Fem	0,018	0,009	Un-Det.	Un-Det.	Un-Det.
2.	M.S	24	Fem	0,012	0,003	Un-Det.	Un-Det.	Un-Det.
3.	L.P	24	Fem.	0,019	0,008	Un-Det.	Un-Det.	Un-Det

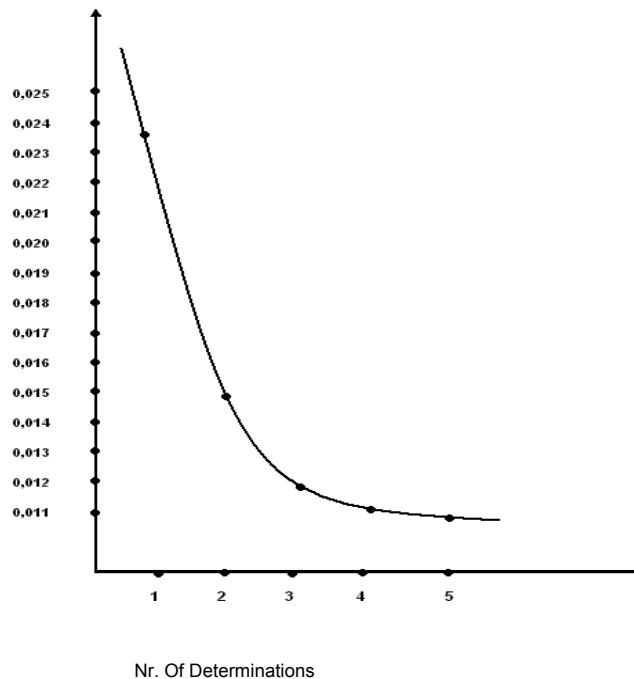
Level of sebum before and after the use of the shampoo in the experimental group.

Nr crt	Case	Age	Sex	Before Treatment (g)	First Day Following Treatment (g)	Second Day Following Treatment (g)	Fourth Day Following Treatment (g)	Following the third washing (g)
1.	P.I	24	Fem.	0,031	0,018	0,016	0,013	0,021
2.	B.M	26	Fem	0,019	0,012	0,013	0,025	0,013
3.	M.C	24	Fem	0,034	0,009	0,009	0,007	0,003

4.	C.P	24	Masc.	0,018	0,009	0,012	0,010	0,014
5.	L.C	25	Masc.	0,034	0,031	0,024	0,017	0,017
6.	V.D	24	Fem	0,018	0,015	0,012	0,012	0,020
7.	P.G	20	Fem	0,030	0,028	0,023	0,018	0,012
8.	L.G	24	Fem	0,021	0,024	0,021	0,016	0,011
9.	D.C	26	Masc	0,022	0,016	0,012	0,014	0,014
10.	P.C	26	Fem	0,031	0,020	0,015	0,012	0,010

We find a decrease in the values, therefore in the level of sebum, in time, the median value for 10 people before treatment: $x = 0,024$ g, after treatment:

- 1st determination: 0.0182 g
- 2nd determination: 0.0157 g
- 3rd determination: 0.014 g
- Last determination: 0.0135 g



After approximately the sixth washing with the anti-seborrheic shampoo, the study group was given an anti-seborrheic lotion. After washing with the shampoo, in the case shown before, and ample rinsing with water, the scalp is rubbed with a cotton swab soaked in the anti-seborrheic lotion.

The level of sebum (the collection being done in the same manner as the collection for shampoo only washing) before and after the use of the lotion is included below:

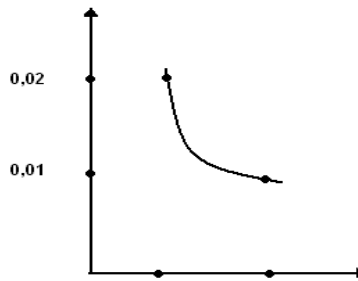
Level of sebum before and after using the lotion – the control group

Nr. crt.	Case	Age	Sex	Sebum level before treatment (g)	Sebum level after treatment – taken on the fourth day (g)
1.	M.V	24	Female	0,012	Un-Det.
2.	C.P	24	Male	0,009	Un-Det.

Level of sebum before and after using the lotion – the experimental group

Nr. crt.	Case	Age	Sex	Sebum level before using the lotion (g)	Sebum level after using the lotion (g)
1.	P.I	24	Fem	0,039	0,009
2.	B.M	26	Fem	0,009	0,009
3.	M.C	24	Fem	0,014	0,012
4.	C.P	24	Male	0,003	0,006
5.	L.C	25	Male	0,102	0,008
6.	V.D	26	Fem	0,010	0,008
7.	P.G	20	Fem	0,004	0,008
8.	L.G	24	Fem	0,008	0,009
9.	D.C	26	Male	0,009	0,003
10.	P.C	26	Fem	0,0012	0,004

A remarkable decrease in sebum level is noticed, from a median level of 0.021 g to that of 0.007 g, approximately 3 times less.



Graphical representation of the median level of sebum before and after using the shampoo combined with the lotion.

II. Biochemical composition of the sebum before and after treatment

The second aspect studied was the determination of the quantity of lipids and of total cholesterol in the sebum collected before and after the treatment. The collected samples were sent to a laboratory for study, where each probe was inserted into an ether-alcohol 1/3 solution and subsequently filtered in order to remove impurities and other products found in suspension.

Filtration was performed on a quantitative filter paper, degreased and soaked in ether-alcohol, followed by an abundant rinsing in warm, with the degreasing mix, in order to collect the eventual bits of fat left on the paper.

The filtered particles were collected and dried in porcelain capsules for a period of 24 hours in a thermo-regulated room. The total lipids (alcohol and soluble ether) were determined gravimetrically on a scale. The samples for which a total cholesterol reading was ordered were measured using the Grigant & Bloor method.

In order to determine the total cholesterol using the Grigant method, the gravimetrically determined lipids were removed from the capsules in small quantities and placed in the warm ether (to collect fat traces) and were thus transferred into a separating Grigant funnel.

Cholesterol was extracted using a 7ml lime alcohol solution (for lathering) afterwards with ether, agitating the solution by tipping it gently. After the two phases (superior by ether and inferior by lime alcohol), the inferior layer was removed, and the etheric phase was washed a few times with distilled water shaking gently (to avoid emulsion) and were then collected in a porcelain capsule as the quantity of ether used. The residue obtained after a 24 hour evaporation process, was added to a 3 ml chloroform solution and put in a glass tubes. The capsule was washed with 2 ml of chloroform taken from the same glass tube. After 20 minutes in the dark, in order to develop the color reaction, a reading was taken with a photocalorimeter using a red filter and a 1 ml cup.

Results noted were weighted against a weighted curve based on a cholesterol solution of 0.06% in chloroform.

Taking into consideration the small quantities of cholesterol from the samples and in order to avoid any other errors, the medium of the 3rd successive reading for each sample in part was repeated at two successive points along the curve and calculated.

The quantity of lipids determined in the sebum samples is noted below in the tables for the experimental group:

Quantity of lipids in the sebum collected from the control group:

Nr crt.	Case	Age	Sex	Before Treatment	First Day Following Treatment	Second Day following 1 washing	Third Day following 1 washing	In time after many washings
1.	I.D	23	Fem	0,016	0,007	Un-Det.	Un-Det.	Un-Det
2.	M.S	24	Fem	0,010	0,001	Un-Det	Un-Det	Un-Det
3.	L.R	24	Masc	0,017	0,006	Un-Det	Un-Det	Un-Det

x x1

Quantity of lipids found in the sebum probes collected from the experimental group:

Nr. crt.	Case	Age	Sex	Before Treatm ent	First Day Following Treatment	Second Day Following Treatment	Third Day Following Treatment	In Time After Many Washings
1.	P.I	24	Fem	0,029	0,016	0,014	0,011	0,019
2	B.M	26	Fem	0,017	0,010	0,001	0,023	0,011
3.	M.C.	24	Fem	0,032	0,007	0,007	0,005	0,001

4.	C.P	24	Male	0,016	0,007	0,010	0,008	0,015
5.	L.C	25	Male	0,032	0,029	0,022	0,015	0,018
6.	V.D	24	Fem	0,016	0,013	0,010	0,010	0,012
7.	P.G	20	Fem	0,028	0,026	0,021	0,016	0,012
8.	L.G	24	Fem	0,019	0,022	0,019	0,014	0,010
9.	D.C	26	Male	0,020	0,014	0,010	0,012	0,009
10.	P.C	26	Fem	0,029	0,018	0,013	0,010	0,008
				X	X1	X2	X3	X4

Quantity of Cholesterol from the collected sebum taken from the control group:

Nr Crt	Case	Age	Sex	Before Treatment	First Day Following Treatment	Second Day Following Treatment	Third Day Following Treatment	In Time After Many Washings
1.	I.D	23	Fem	4,8	Un-Det	Un-Det	Un-Det	Un-Det
2.	M.S	24	Fem	2,3	Un-Det	Un-Det	Un-Det	Un-Det
3	L.P	24	Fem	5,1	Un-Det	Un-Det	Un-Det	Un-Det

$$X=4,07$$

Quantity of total cholesterol in the sebum collected from the experimental group:

Nr Crt	Case	Age	Sex	Before Treatment	First Day Following Treatment	Second Day Following Treatment	Third Day Following Treatment	In Time After Many Washings
1.	P.I	24	Fem	5,5	3,04	2,1	1,8	3,6
2.	B.M	3,2	Fem	3,2	1,9	1,8	4,1	1,8
3	M.C	24	Fem	6	1,38	1,33	1,18	0,19
4.	C.P	24	Male	3	1,33	1,9	1,2	2,3
5.	L.C	25	Male	6	5,5	4,1	2,3	2
6.	V.D	24	Fem	3	2,47	1,9	1,9	1,6
7.	P.G	20	Fem	5,3	4,9	4	3,04	3
8.	L.G	24	Fem	3,6	4,1	3,6	2,1	1,6
9.	D.C	26	Male	3,8	2,1	1,9	2,5	1,7
10.	P.C	26	Fem	5,5	2,8	2,47	1,9	1,6
				x=4,4	X1=2,9	X2=2,5	X3=2,22	X4=2,03

III. Determining the microbial flora before and after the use of Acnistat - Shampoo

The samples collected were sent to a microbiology lab where from the quantity of the samples, seeding was performed on a culture media (glucose bullion and gelled blood in Petri dishes of 10 cm diameter) in order to determine the total microbial flora.

On the culture media, before the application of the treatment, non-pathogenic germs (saprophytes such as Corynebacterium, Phytosporum ovale, Coagulase-negative Staphylococcus, Streptococci) as well as pathogenic germs (Coagulase-Positive).

After using the anti-seborrheic preparation, the number of calories is reduced significantly, as well as the variety of germs is reduced (there remains Coagulase-negative Staphylococcus, Corynebacterium).

According to these results, certain subjective modifications occurring after the treatment, such as the disappearance of pruritus. It has been covered in medical literature that Propionibacterium and Staphylococcus are capable of synthesizing certain enzymes: lipase, phosphatase, neuraminidase, hyaluronidases, lecithinase. The type of enzyme released together with the biological activity varies in function according to the physio-chemical conditions of the culture medium.

These enzymes hydrolyze the lipids and determine the formation of fatty acids with irritating effects.

IV. Particular subjective aspects

Observations made by subjects in the experimental group before treatment:

- intense pruritus of the scalp, appearing shortly after washing with a normal shampoo
- wet seborrhea represented by a shiny look, oily hair, by an uncomfortable sensation of hairs sticking together and the feeling that the scalp is oily and full of particles. These observations were found primarily in case no 1, 7, and 8 of the experimental group, which related that maintaining a 4 – 5 day interval between washings was difficult.
- Dried seborrhea appears as white particles at the scalp's surface
- More intense hair loss generally appears during the washing and combing / brushing of the hair.

After using the shampoo and afterwards the associated lotion, a feeling of comfort and lightening of the scalp was felt. There was a disappearance of pruritus a prolonged interval in between two washings of 10, 11 days and a complete stopping of hair loss.

Acnistat – Shampoo and Antiseborrheic Lotion

Acnistat – Anti-Seborrheic Shampoo is made entirely out of natural plant extracts containing a series of bio-hormones which act as local anti-androgens. In this manner, the action of the androgenic hormones is prevented on the scalp of patients. The clinical effect is extremely important – following a month or a month and a half of treatment, the results are spectacular. The absence of dandruff and the halting of hair loss are effects which are not obtained using other shampoos.

Precautions: Please avoid contact with eyes. If shampoo gets into the eyes, please rinse with water abundantly. Avoid getting shampoo in your inner ear canal.

Ingredients: aqua, sodium laureth sulfate, cocamidopropyl betaine, sodium chloride, cocamide Dea, Calendula officinalis, aloe barbadensis, tocopheryl acetate, Disodium EDTA, Polyquaternium-7, Zinc Pyrithione, Imidazolidinyl Urea, Parfum, C.I. 42510.

Please shake both the shampoo and lotion well before use.

It is recommended washing the hair twice a week.

1. Following the soaking of one's hair, apply 3-4 ml (half a lid) shampoo to scalp
2. Massage intensely for 5 minutes in the scalp in order for the active substances to penetrate the roots of the hair.
3. Wait around 10 minutes in order for the substances to activate.
4. Rinse well and repeat
5. After around 2 days, apply anti-seborrheic lotion to the scalp.

Adverse effects: People which experience a particularly raised sensitivity or allergies to different substances, Acnistat anti-seborrheic Shampoo, can theoretically cause minimal allergic reactions, manifested by reddening of the scalp and itchiness, during medical testing no such reactions have been found. Abundant washing with water to remove all shampoo traces is indicated in these situations.

Packaging: 200 ml bottles

Shelf Life: 36 months

Approved by the Ministry of Health – Notice No. 47999/13.05.2008