

Study of Vasodilating and Regenerative Effect of the Gel with Nettle Juice intended for Telogen Effluvium Treatment

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ABSTRACT

Telogen effluvium remains the most common diffuse hair loss in women which influences the quality of their life. Stinging nettle leaves and stems contain a wide range of biologically active substances which stimulate the metabolism and trophic processes in hair follicle cells. The purpose of the research was to study the vasodilating effect and follicle-stimulating activity of the gel with nettle juice in rats under conditions of wool shedding induced by orally administered boric acid. Thus, conducted pharmacological studies indicated that the gel in comparison with the untreated animals accelerated the wool growth in rats, improved its quality by increasing the weight and reducing the percentage of dystrophic hairs. Histological studies confirmed that cutaneous application of the gel significantly dilated the vessels in the dermis reticular layer. The intensification of blood supply was confirmed by an increase in the number of mast cells and their active degranulation. Strengthening of blood circulation caused the increase of the hair follicle quantity in the active anagen phase.

INTRODUCTION

The prevention and treatment of Telogen effluvium (TE) remains the important task of modern medicine. TE is the most common diffuse hair loss in women of reproductive age, which is due to concomitant somatic pathology (disorders and diseases of the gastrointestinal tract and the endocrine system, infectious diseases, etc.) and/or negative exogenous factors (inappropriate nutrition, drug intoxication, stress, inappropriate hair care, etc.) (Shrivastava, 2009). Hair follicles (HF) affected by harmful factors are characterized with anagen (active growth) termination, which results in the onset of catagen (hair involution) and subsequently 2-3 months telogen (resting phase) finishing with exogen – dead hair shedding (Figure 1) (Harrison and Bergfeld, 2009).

In the TE therapy plants remedies occupy the important

place (Patil *et al.*, 2010; Kaushik *et al.*, 2011). Plant biologically active substances (BAS) with vasodilation, capillary protective, epithelial cells regenerating effects, microelements, amino acids, vitamins and other nutrients for HF tissues saturation are widely used (Lourith and Kanlayavattanukul, 2013; Semwal *et al.*, 2015).

From olden times in medicine and cosmetology stinging nettle (*Urtica dioica* L.) was used for renovation and hair growth stimulation. Nettle leaves and stems contain a wide range of BAS: organic (formic, pantothenic) and hydroxy cinnamic (chlorogenic, caffeic, ferulic) acid, flavonoids (rutin, quercetin, campherol), chlorophyll, organically bound silicon, a complex of vitamins (K₁, carotenoids, vitamins C, B₁, B₂, B₆, etc.) and other BAS (amines, amino acids, macro and microelements). The listed BAS enhance blood circulation in the skin capillary system, stimulate the metabolism and trophic processes, exhibit regenerative properties of HF cells, therefore the remedies with stinging nettle (infusions, tinctures, lotions, masks, juices, etc.) are used for the TE prevention and treatment (Gülçin *et al.*, 2004; Bhuwan *et al.*, 2014; Mal Ait Haj Said *et al.*, 2015).

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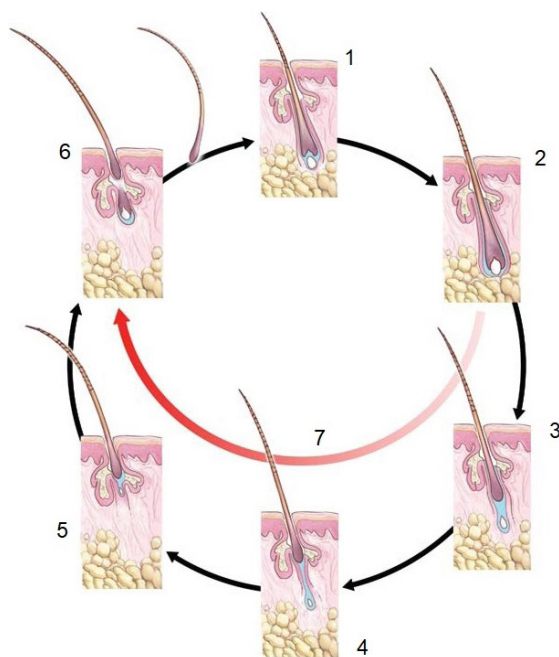


Fig. 1: The hair cycle and telogen effluvium (Harrison and Bergfeld, 2009; medical illustrator: William Garriott). 1,2 – anagen phase (active hair growth, 2-8 years), 3,4 – catagen phase (involution, 4-6 weeks), 5 – telogen phase (resting, 2-3 months), 6 – exogen phase (release of dead hair), 7 – telogen effluvium (anagen termination, noticeable increase in hair shedding occurs 2 to 3 months after hair was affected).

The technological, rheological and microbiological studies were carried out, which resulted in the development of the medical cosmetic gel with nettle juice intended for TE therapy (Fedorovska *et al.*, 2015; Fedorovska and Polovko, 2016). Purpose of the given research was to study the vasodilating effect and follicle-stimulating activity of the developed gel with nettle juice in rats under conditions of wool shedding induced by orally administered boric acid (Belenichev *et al.*, 2008).

MATERIAL AND METHODS

Preparation of nettle juice

Nettle fresh upper parts of the stems with 5-6 leaves were collected at the end of May in Ivano-Frankivsk region (Ukraine). Technological process of nettle juice preparation included the following stages: grinding of fresh raw materials, pressing, re-grinding and pressing, inactivation of enzymes in raw juice with 20 min heating in a water bath at 40-60°C, preservation with 96% ethanol in quantity of 15% by weight, sedimentation at 2-4°C in a refrigerator for 10 days, filtration, packaging, labeling. Obtained juice was a dark brown transparent liquid with a specific smell; density ranging from 0.994 to 1.106 g/ml; pH from 6.5 to 7.0; dry residue at least 3.5%; identification (thin layer chromatography method) and quantitative determination (spectrophotometric method): hydroxyl cinnamic acids at least 0.01%, carotenoids at least 0.002%, chlorophyll at least 0.003% (Fedorovska *et al.*, 2015).

Preparation of gel with nettle juice

The remedy containing 15% per weight of the nettle juice in a gel base was prepared. Briefly, combine carbopol-so-

dium alginate gel base was prepared firstly. Potassium sorbate was used as a carbopol neutralizer as well as a gel preservative, glycerol was used as a sodium alginate solubilizer as well as a moisturizer, purified water was used as a solvent (Fedorovska and Polovko, 2016). After weighing, the nettle juice was added in the gel base by continuous mixing at room temperature.

Experimental animals

White non-linear rats of both sexes with weight range of 200-250 g were obtained from the vivarium of Ivano-Frankivsk State Medical University. All experimental protocols were approved by the Ethical Committee of the University (Minutes No. 69/13, dated 2013.12.03). Animals were kept under standard vivarium conditions, they received food and water *ad libitum*.

Experimental design

All 28 rats were weighed and tagged; growth destruction of animal wool was induced with oral administration of boric acid (5 mg/kg) during 14 days, then wool was completely shaved on the back section (3 × 7 cm). After that all rats were randomly divided into four groups of seven animals each as followed: 1st group – intact animals; 2nd group – untreated; 3rd group – treated with a comparison drug (Alloton spray: complex tincture (1:5) from a mixture of *Arctium lappa* L. roots – 5.6 g, *Sophora japonica* L. fruit – 5.6 g, *Acorus calamus* L. rhizome – 3.2 g, *Urtica dioica* L. leaves – 2.8 g; *Humulus lupulus* cones – 2.8 g); 4th group – treated with the prepared gel. The developed medical cosmetic remedy and the comparison drug were applied on the shaved back of the rats at the maximum technically spreading dose (app. 1.0 g of the developed gel; 3-4 pulverizations of Alloton spray) during next 14 days.

Analytical methods

According to the experiment, the wool length was daily monitored during 14 days. After that period, new wool was completely cut and weighed on scales. The percentage of dystrophic wool (presence of hairs with pointed tip) was observed and determined with help of a microscope “Delta Optical Genetic Pro” with built-in camera: lens 40/0.65 160/0.17; eyepiece WF 10×/18). Additionally, from each group one animal was randomly selected and subjected to euthanasia with compliance of the bioethics; skin biopsy was applied to the chosen animals. The vascular net in different layers of the skin, follicular density (number of follicles per unit area) and hair growth phase (anagen) were estimated.

The study of the vascular net was carried out by the injection method using an ether-chloroform mixture of Paris blue (10 g of substance per 100 ml of a solvent consisting of ether and chloroform in a ratio of 3:1). Fuchseline-picrofuchseline was used for histological examination of the vascular wall, the study of skin structures was carried out with the hematoxylin and eosin staining. The number of hair follicles was determined by morphometric analysis. All histological experiments were performed at the Human Anatomy Department of Ivano-Frankivsk State Medical University according to standard techniques (Bagrij, 2016; Titford, 2009; Kiernan, 2015). The electronic microscopic photographs were made with an electron microscope PEM-125 K at magnifications of 8000-10000 and an accelerating voltage of 75 kV with subsequent photographing; microscopic photo-

graphs were made with the microscope “Delta Optical Genetic Pro”.

Statistical analysis

The data of external estimations (given in Tables 1-2) were analyzed as the mean \pm SEM for seven animals in each group. The data of histological studies (vascular lumen diameter, the number of hair follicles per 1 mm²) were studied as the mean \pm SEM for ten skin samples of each selected animal. Statistical analyses were performed using one-way analysis of variance (ANOVA). Differences were considered to be statistically significant when P was < 0.05 . All statistical analyses were performed with MS Excel.

RESULTS AND DISCUSSION

The results of the developed gel influence on the growth stimulating activity of the rats' wool are presented in Tables 1-2.

According with the data in Table 1, the developed gel stimulated the recovery of damaged HF growth and slightly exceeded “Alloton” in terms of regenerative action. Thus, wool began to grow on the 3rd day of the experiment in the 1st, 3rd and 4th groups, but in the 2nd group (untreated animals) – on the 4th day. Intensive wool growth in the intact (1st group) and the treated (3rd and 4th group) animals was observed on the 5-8th days of the research. On the 14th day of the experiment, the difference in the wool length in the experimental 4th group was exceeded 3 mm in comparison with the untreated 2nd group.

On the 15th day of the experiment (Table 2) the wool weight from the shaved area of 4th group (experimental animals) was ≈ 480 mg, which significantly exceeded the wool weight of the non-treated 2nd group (≈ 371 mg). The wool weight of the healthy animals in the 1st group was slightly higher (≈ 500 mg), and in the 3rd group (rats treated with “Alloton”) at approximately the same level (≈ 472). The experimental 4th group also showed a decrease in the percentage of dystrophic wool compared to the untreated

animals. So the amount of dystrophic wool in the healthy animals was $\approx 10\%$, in the experimental 4th group – $\approx 17\%$, and in the untreated animals – $\approx 38\%$ (Table 2).

Table 1: The influence of the developed gel with nettle juice on the experimental animal wool growth (mm).

Day	Intact rats (1 st group)	Untreated rats (2 nd group)	Rats treated with “Alloton” (3 rd group)	Rats treated with developed gel (4 th group)
2	-	-	-	-
3	1.0 \pm 0.07	-	1.0 \pm 0.06	1.0 \pm 0.05
4	2.0 \pm 0.07	1.0 \pm 0.07	1.8 \pm 0.06	2.0 \pm 0.04
5	3.1 \pm 0.07	1.9 \pm 0.01	2.7 \pm 0.06	3.1 \pm 0.04
8	6.6 \pm 0.06	3.7 \pm 0.10	6.4 \pm 0.01	6.7 \pm 0.06
10	7.7 \pm 0.07	5.3 \pm 0.07	7.2 \pm 0.06	7.8 \pm 0.04
14	9.8 \pm 0.05	6.5 \pm 0.05	8.9 \pm 0.05	9.7 \pm 0.06

* – ($p < 0.05$) in relation to the 2nd group (untreated rats).

Table 2: Influence of the developed gel on the quality and structure of the rat wool.

Characteristic	Intact rats (1 st group)	Untreated rats (2 nd group)	Rats treated with “Alloton” (3 rd group)	Rats treated with developed gel (4 th group)
Wool weight from shaved area, mg	499.2 \pm 6.8	370.5 \pm 9.8	472.0 \pm 8.7	480.3 \pm 9.3*
% of the dystrophic wool	10.32 \pm 0.38	37.72 \pm 2.15	19.51 \pm 1.05	16.87 \pm 0.90*

* – ($p < 0.05$) in relation to the 2nd group (untreated rats).

The performed studies confirmed that the external application of the developed gel caused the significant changes in both histological and ultrastructural organization of subcapillary and dermal arterial nets. However, changes in the dermal arterial network were more distinct and accompanied by enlargement of the capillary lumen and by the vascular wall thickening (Figure 2).

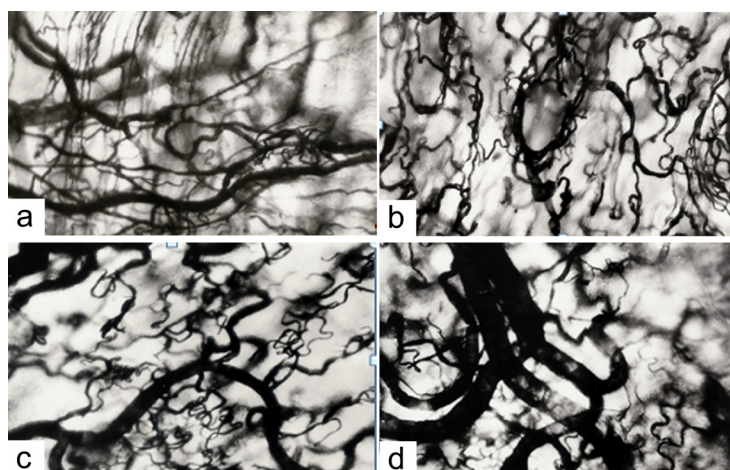


Fig. 2: Dermal arterial net of white rats' skin samples: a – 1st group; b – 2nd group; c – 3rd group; d – 4th group. Vessels injection with Paris blue; an increase in 400.

The widest vascular lumen (diameter = 76.45 ± 0.42 μm) was in the skin samples of the 4th group after developed gel application (Figure 2 d). The vessels in the skin samples of 1st

group had a diameter of 69.85 ± 0.25 μm (Figure 2 a). The skin of animals treated with “Alloton” was characterized by a less distinct vascular pattern (Figure 2 c).

Near the dermal vessels of the 4th experimental group the number of mast cells significantly increased, which were in a state of strong degranulation (Figure 3). Particularly strongly degranulated mast cells were seen in the reticular layer of the dermis. On the ultrastructure level it was observed a significant decrease of the mast cells size and granules that went beyond their edges (Figure 3 b). High degranulation of mast cells and their functional activity is a factor of changes in blood vessels, which indicate the intensification of blood circulation in the skin.

When applying the gel with nettle juice, a significant increase in the number of new hair follicles was noted (Figure 4).

The number of follicles in the stage of anagen in skin

samples of 4th group was 93% (compared with: 1st group – 87%; 2nd group – 74%; 3rd group – 88%). Also, the intensification of the HF epithelial cells proliferative activity was observed (Figure 5).

HF density per unit area (1 mm²) in the skin samples was determined with the morphometric analysis. The HF number in the 1st group was 36.34 ± 0.14 , in the 2nd group – 21.98 ± 0.23 , in the 3rd group – 34.56 ± 0.21 , in the 4th group – 37.23 ± 0.19 . As can be seen from the study results, the application of the developed gel in the experimental animals strongly stimulated HF genesis compared to the untreated rats. The samples of rats' skin tissues from each group are represented in Figure 6 for visual comparison of the HF quantity.

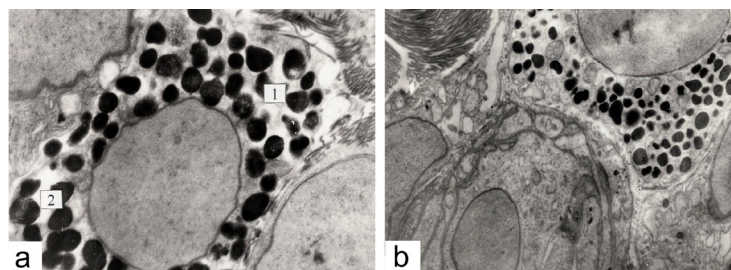


Fig. 3: Ultrastructure of the rats' skin mastocytes in the dermal papillary layer (1, 2 – granules of different maturity degrees): a – 1st group; b – 4th group. Electronic microscopic photographs; an increase in 10000.

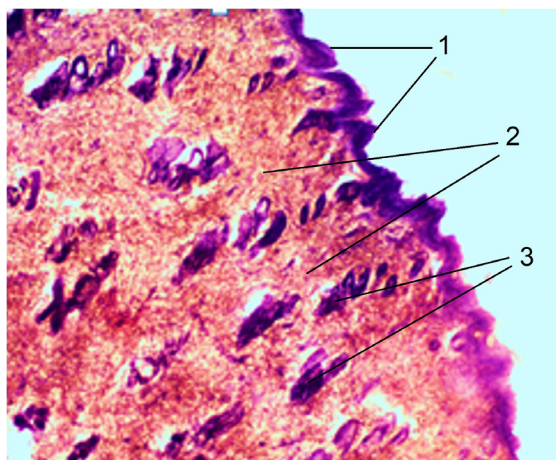


Fig. 4: A significant amount of hair follicles in the skin of the white rat after the application of gel with nettle juice: 1 – epidermis, 2 – dermis, 3 – hair follicles. Coloring with hematoxylin and eosin; microscopic photograph; an increase in 100.

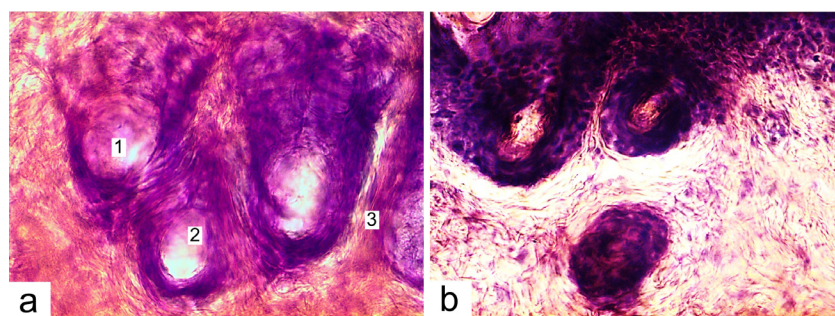


Fig. 5: The structure of the outer and inner epithelial sheath of the white rat HF (1 – root, 2 – internal epithelial sheath, 3 – external epithelial sheath): a – 1st group; b – 4th group. Coloring with hematoxylin and eosin; microscopic photographs; an increase in 400.

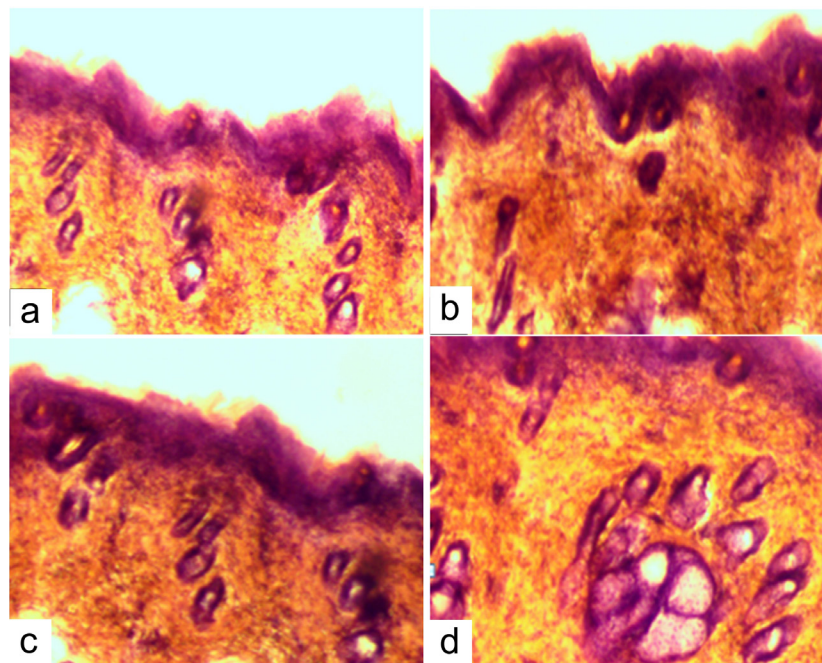


Fig. 6: Hair follicles in the skin of white rats: a – 1st group; b – 2nd group; c – 3rd group; d – 4th group. Coloring with hematoxylin and eosin; microscopic photographs; an increase in 200.

CONCLUSION

Thus, conducted pharmacological and histological studies indicated that the developed gel with nettle juice in comparison with the untreated animals accelerated the wool growth renovation in rats, improved its quality by increasing the weight and reducing the percentage of dystrophic hairs.

It was proved that cutaneous application of the gel had biological effect that significantly dilated the vessels in the reticular layer of the dermis. The intensification of HF blood supply was confirmed by an increase in the number of mast cells and their active degranulation. Strengthening of blood circulation caused the increase of the epithelial cells growth and HF number in the active phase (anagen).

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