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THE UVB-SHIELDING EFFECT OF A MAGNETITE NANOPARTICLE CREAM AFTER 28 DAYS OF USAGE IN A MURINE MODEL: A SKIN'S HISTOARCHITECTURE STUDY

EFECTUL ECRANĂRII UVB AL UNEI CREME CU NANOPARTICULE DE MAGNETIT DUPĂ UTILIZAREA TIMP DE 28 DE ZILE ÎNTR-UN MODEL MURIN: UN STUDIU AL HISTOARHITECTURII PIELII

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ABSTRACT | REZUMAT

A promising and effective combination with magnetic nanoparticles, possessing UVB protective properties, is the lanolin-based ointment containing magnetite nanoparticles (Fe_3O_4). Two experiments were conducted on 42 BALB/*c* albino mice, with two objectives: initially, to evaluate the UV protective effect of a magnetite nanoparticle ointment at 6G (6.96 mg/g lanolin) and 12G (13.92 mg/g lanolin), compared to a zinc oxide (ZnO_2)-based ointment, and to follow, the stadial protective UV screening effect of magnetite nanoparticles, in a 28-day-study, (at 7, 14, 21, and 28 days). After applying the screening agent (6G), the animals were exposed to a UV III/A-B lamp, with UVB wavelengths, middle UV (280-320 nm, photon energy = 3.94−4.43 eV), for 120 min./E1 and 30 min./day/E2; then ear skin samples, stained H&E and Perls, were histologically investigated (at 100, 200, and 400 ×). The histological findings proved that 6G magnetic nanoparticles were the appropriate dose, in terms of handling, adeptness, providing an UV-protective long-time effect, and not causing harmful changes in the skin, within repeated applications. The lanolin-based nanocomposite with 6G magnetite particles can constitute an effective physical UV protective agent through aggressive light radiations.

Keywords: magnetite nanoparticles, murine model, skin histology, UV

O combinație promițătoare și eficientă cu nanoparticule magnetice care posedă proprietăți de protecție UVB este unguentul pe bază de lanolină care conține nanoparticule de magnetit (Fe3O4). Au fost efectuate două experimente pe 42 de șoareci albino BALB/c cu două obiective: evaluarea efectului protector UV al unui unguent cu nanoparticule de magnetit la 6G (6,96 mg/g lanolină) și 12G (13,92 mg/g lanolină) comparativ cu unguentul pe bază de oxid de zinc (ZnO2) și urmărirea efectului protector de ecranare UV al nanoparticulelor de magnetit într-un studiu de 28 de zile (la 7, 14, 21 și 28 de zile). După aplicarea agentului de examinare (6G), animalele au fost expuse la o lampă UV III/A-B cu lungimi de undă UVB (280-320 nm, energie foton = 3,94−4,43 eV) timp de 120 min./E1 și 30 min./zi/E2. Probele recoltate au fost investigate histologic (la 100, 200 și 400 ×). Constatările histologice au demonstrat că nanoparticulele magnetice 6G au reprezentat doza adecvată din punct de vedere al manipulării, al aderenței, al efectului de protecție UV pe termen lung și al faptului că nu au provocat modificări nocive ale pielii, în cadrul aplicațiilor repetate. Nanocompozitul pe bază de lanolină cu nanoparticule de magnetit la concentrația de 6G poate constitui un agent fizic protector eficient împotriva radiațiilor UV agresive.

Cuvinte cheie: nanoparticule de magnetit, model murin, histologie cutanată, UV

Magnetic materials are distinct particle characterised by exceptional attributes that can be utilised in various technologies, including biomedical applications. Among these magnetic nanomaterials, systems based on iron (II, III) oxide (Fe_3O_4, magnetite) are intensely studied (13, 27). Iron oxide is the most investigated as authorised nanomedicine. At given diameters, from 15 nm but no more than 100 nm, magnetic iron oxide NPs (MNPs), which are made up of magnetite (Fe_3O_4) or maghemite (Fe_2O_3), confirmed to be efficient in multifarious domains. This is why the substance is seen as exceptionally promising in the biomedical applications (e.g., in diagnostics, imaging, or in the photothermal therapies) (19).

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Persistent exposure to sunlight is recognised to have detrimental outcomes on human skin. Presently, extensive research in dermato-medicine is targeting the development of effective strategies for alleviating the adverse aftereffects of lengthened exposure to solar radiation (6). Various sectors, such as agriculture, military, sports, construction, and navigation, involve prolonged daily exposure to sun and solar radiation. Among individuals susceptible to skin cancer, farmers rank highest, due to their prolonged outdoor exposure to solar radiation during usual workdays (22, 33).

The harmful effect on the skin resulting from prolonged and unprotected exposure to UV radiation is well known, with the clinical and histological aspects induced in the skin being described in detail. Additionally, it has been established that the persistence of erythema is dependent on the wavelength of radiation (1, 38).

Therefore, erythema generated by UVB and UVA,

which penetrate the dermis (UVB in the upper portion and UVA in the deep dermis), persists for several days. Compared to UVA radiation, UVB radiation is the one that produces the most severe effects on the skin. Additionally, numerous observations show that exposure to unprotected UVR (ultraviolet radiation) alters the immune response and origins numerous forms of skin tumours. Nevertheless, the long-term effects on the skin remain inadequately understood, necessitating comprehensive studies (16, 38).

Thus, for example, a prolonged exposure to UVR induces an imbalance in the homeostasis of free oxygen radicals, which represents the first step in a long chain of chemical reactions in the installation of photolesions on the skin, with echoes on the whole body.

Although the body has enzymatic (antioxidants such as glutathione peroxidase, catalases, superoxide dismutase, thioredoxin, and peroxiredoxin) and non-enzymatic (antioxidants such as carotenoids, resveratrol, quercetin, ellagic acid, vitamins C and E) levers to counter the negative effects of reactive oxygen species, their direct or indirect action causes damage to DNA molecules through the selective oxidation of guanine, dimerisation of thymine, and promoting abnormal interactions between nucleotide bases (18, 25).

Under the action of UVR, through the production of reactive oxygen species, not only the cellular DNA suffers, but also the mitochondrial one, the two phenomena inducing the triggering of exogenous (through the activation of surface receptors for cell death) and endogenous (the imbalance between the family of preapoptotic, such as Bax, Bak, and Bid, and that of antiapoptotic proteins, such as Bcl2 and Bcl-x), which, through the programmed suicide of cells, prevents the installation of inflammatory phenomena and those of cellular malignancy. DNA damage is physically expressed at the skin level by the installation of acute lesions, manifested by sunburn, erythema, painful oedema and photodermatoses, and/or by the installation of chronic conditions such as photoaging, hyperpigmentation, and precancerous lesions, which can culminate in the installation of actinic keratosis and cancer, which can affect the basal cells of the epidermis (basal cell carcinoma), keratinocytes (squamous cell carcinoma) or, much more seriously, melanocytes (malignant melanoma) (36). Reactive oxygen species also act indirectly on the synthesis of new collagen and elastic fibres, glycosaminoglycans, and adhesion molecules of the connective extracellular matrix. These malfunctions of the connective tissue led to the installation of inflammatory phenomena, which can be expressed through photoallergies and photodermatitis. However, with all these negative effects of exposure to UVR, one cannot ignore its main beneficial effect, namely the synthesis of vitamin D, which is responsible for the homeostasis of calcium (which is also the second-order messenger of cellular signalling) and phosphorus in the body, involving - it is also involved in other processes such as hormone synthesis, cell differentiation and proliferation, and immune reactions. Many conditions today are linked to vitamin D deficiency, starting with infectious diseases, diabetes, obesity, and heart problems and ending with various forms of cancer and autoimmune diseases (18, 25, 36).

In recent years, there has been a widespread adoption of UV-protective physical agents. Chemical sunscreen agents are less commonly used and recommended because of the harmful effects they can cause on the skin, especially on the body in general. Compared to chemical agents used as UV sunscreen agents, physical shielding agents have a number of advantages (8, 21, 26). For example, chemical agents, such as octyl salicylate or octyl methoxycinnamate in hybrid lipid-silica nanoparticles, proved to be innovatively used in sunscreens to specifically absorb UVB radiation and convert it into heat (2). Presently, extensive research in human and veterinary science endeavours aims to develop effective strategies for mitigating the adverse consequences stemming from prolonged exposure to solar radiation.A number of studies conducted in recent years observed that certain types of nanocomposites with magnetic particles can be used successfully as sunscreens. Physical sunscreens based on zinc oxide and titanium dioxide are currently used, but the appearance of these products is less aesthetic (29, 35). By comparison, nanocomposites with magnetic particles (magnetite, cobalt ferrite), in addition to the remarkable effects as physical shielding agents, also possess special aesthetic qualities, giving the skin treated with these products a pleasant, slightly coppery appearance (28).

Another advantage of magnetic particle nanocomposites is their stability over time, even years, during which the uniform dispersion of nanoparticles in the compounds used is maintained. This argument recommends that they be used in various fields as UV protection screens (31).

The category of physical sunscreens acts by absorbing and scattering UV radiation. Chemical sunscreens rely solely on the absorption of UVB and conversion into heat. The more widely spread and preferred protective screens are those that utilise both categories of factors, both physical and chemical (5).

If all these effects are known, however, researchers and communities are still faced with two major issues of interest to which they must respond and find solutions. Firstly, if these harmful effects induced in the body can be alleviated and how, and secondly, if the changes produced in the body by UVR can be prevented and how (17). Based on the results from previous experiments conducted in murine models, it was observed that a promising and effective combination with magnetic nanoparticles, possessing UVB protective properties, is the lanolin-based ointment containing magnetite nanoparticles (Fe_3O_4).

MATERIALS AND METHODS

The investigations took into account two objectives in a murine model:

Experiment 1 - to evaluate the UV protective effect of an ointment containing magnetite nanoparticles compared to zinc oxide (ZnO_2)-based ointment, and

Experiment 2 - to follow the protective UV screening effect of magnetite nanoparticles in a 28-day study.

The animals and experimental protocol

Ethics Commission approbation

The experiment was approved by the Ethics Committee of the Faculty of Veterinary Medicine from the University of Life Sciences "*King Michael I*" from Timisoara (no. 136/2021), in accordance with all domains' national and international relevant regulations (9).

Animals and experimental protocol

The experiments were conducted on a total of 42 individuals (n = 18 / E1; total, n = 24 / E2), randomly distributed, ageing 3 months, with a mean weight of 26 ± 2g, males and females, of BALB/*c* albino mice. The study was completed in triplicate, repeated at one-week distance (n = 2 / replication × 3 = 6 individuals / Group) as presented in Fig.1.

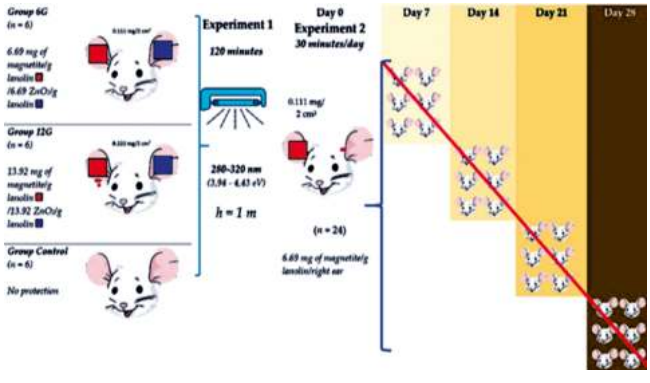

Fig. 1. Animals and experimental protocol

Experiment 1

Group G6 (n = 6) – lanolin-based magnetic ointment at a concentration of 6G was applied to the right auricular pavilion (both on the inner and outer sides), while the left ear was treated with zinc oxide-based ointment at the same concentration (6.96 mg ZnO_2/g lanolin);

Group 12G (n = 6) – the concentration of 12G was applied in the same way as for G6, at a concentration of 13.92 mg ZnO_2/g lanolin;

Group Control (n = 6) – control was non-protected and UV exposed only.

On a measured auricular surface of 2 cm^2, one drop (0.016 mL) of each type of screening agent was applied. Therefore, a concentration of 0.111 mg and 0.222 mg of nanoparticles per 2 cm^2 of skin surface was applied. For the zinc oxide cream, the dispersion of particles was similar to magnetic ointment (0.111 mg and 0.222 mg / 2 cm^2). After applying the screening agents, the animals were exposed to irradiation using a UV III/A-B lamp with UVB wavelengths, middle UV (*Dorno radiation*), ranging 280-320 nm (photon energy 3.94–4.43 *e*V). Animals were placed 120 minutes under the lamp at a 1-meter distance to achieve 100% relative energy.

Experiment 2

Followed the protective effect of lanolin-based ointment with 6G magnetite nanoparticles for 28 daily applications and 30 minutes of UV radiation/day. The screening agents were applied to the auricular pavilions, as follows:

Group G6 (n = 24) – ointment 6G was applied to the right auricular pavilion (inner and outer sides), and a drop (0.016 mL) of nanocompound containing 0.111 mg of magnetite nanoparticles was applied, while the left auricular pavilion of mice was considered Control

Table 1

Staining protocol used

Staining method		
Phase succession	**Method protocol**	**Time**
Haematoxylin-Eosin		
Deparaffinization	in two benzene baths	20 minutes - each
Hydration	two baths in solutions with decreasing concentrations of absolute ethanol: 96°, 80°, 70°	10 min. each/concentration
Staining the nuclei	with Harris haematoxylin	3 minutes
Wash & differentiation	in tap water	2 minutes
Staining the cytoplasm	2% eosin	3 to 5 minutes
Dehydration	in increasing concentrations of absolute ethanol: 70°, 80°, 96°	10 min. each/concentration
Clarification	in two benzene baths	15 minutes each
Mounting	in Canada balsam	1 minute
Perls - for highlighting iron		
Deparaffinization	in two benzene baths	20 minutes - each
Hydration	two baths - in solutions with decreasing concentrations of absolute ethanol: 96°, 80°, 70°	10 min. each/concentration
Rinse 1	in distilled water	5 minutes
Immersion	in Perls reagent	20-30 minutes
Staining	with neutral red	2 minutes
Rinse 2	in distilled water	5 minutes
Dehydration	in increasing concentrations of absolute ethanol: 70°, 80°, 96°.	10 min. each/concentration
Clarification,	two benzene baths	15 minutes each
Mounting.	In Canada balsam	1 minute

and not protected.

During the experiment, after 7, 14, 21, and 28 days of exposure, six albino mice per group were sacrificed ($n = 6 = 3 \times 2$) to analyse the histology of the skin.

The magnetic ointments formulation

The tested ointment was prepared, with magnetite nanoparticles in the Laboratory of Magnetic Fluids, from the Centre for Fundamental and Advanced Technical Research of Romanian Academy Timisoara Branch, Romania. The biochemical and biophysical characterisation was made and presented in earlier articles. The preferred size of magnetic nanoparticles, considered to be safest for the cells, was a spherical shape of 10-15 nm.

To establish the most appropriate dose of nanoparticles with a UV-protective effect, there were obtained two concentrations: lanolin-based ointment (*Elemental, Romania*) with magnetic particles at concentrations of 6G and 12G.

The ointment 6G corresponded to a concentration of 6.96 mg of magnetite nanoparticles/gram of lanolin, while the 12G ointment had a concentration of 13.92 mg of magnetite nanoparticles/gram of lanolin. The lanolin base was stabilised and purified in beeswax (*Elemental, Romania*), which gave structure, firmness, and emollient benefits to the ointment.

To comparatively highlight the UVB-protective effect of the nano-compounds, a usually used screening agent, ZnO_2 ultra-pure grade powder (*Mayam, Romania*), was also applied in a lanolin-based ointment.

Histology and samples colouration

The auricular pavilions were collected and processed as presented in Table 1.

Slices of 5 mm were sectioned on a Cut-4062 microtome (Mainz, Germany),mounted on slides, and stained with haematoxylin-eosin (H&E) and the Perls method (considered with a high-level specificity in revealing iron in the skin) (7). Microscopy was performed at ×100, 200, and 400 µm using an Olympus CX41 microscope (Olympus, Hamburg, Germany), which included a digital camera and QuickPhoto-Micro2.2 software (Promicra, Prague, Czech Republic).

RESULTS AND DISCUSSION

Experiment 1

After the exposure period ended, the clinical examination of the auricular pavilions on the white mouse serving as a control revealed the presence of very pronounced primary erythema, typical of UV irradiation.

In the case of mice treated with zinc oxide, the clinical examination showed the appearance of small and scattered erythema, much less pronounced compared to the control. However, these macroscopic skin changes in the ear region were much more evident in the case of protection with ointment containing zinc oxide at a concentration of 6.96 mg/g lanolin compared (6G) to the higher concentration of 13.92 mg/g lanolin (12 G). In the case of albino mice treated with magnetic ointment, no primary erythema were observed after two hours of exposure to ultraviolet radiation.

Histological findings

Microscopic examination of sections taken through the control auricular pavilion in Experiment 1 is revealed in Fig. 2. The presence of cellular vacuolisations, heterochromatinizations of nuclei in the spinous layer, and degenerations of the basal membrane, as observed through both Perls staining and the haematoxylin-eosin technique. Furthermore, there is noted a destruction over large areas of the basement membrane that separates the papillary dermis from the epidermis.

The destruction of this membrane and the creation of spaces between the epidermis and dermis constitute a predisposing factor for the development of basal and squamous cell skin cancers (Fig. 2 a, b).

Degenerative phenomena have also been reported at the level of hair follicles. Additionally, most sebaceous glands exhibit almost complete necrosis of secretory cells and, to a large extent, of proliferative basal cells. Thus, the amounts of sebum produced are considerably reduced, a phenomenon that explains the drying and fragility of the skin in cases of irradiation. In the microscopic examination of sections taken through the auricular pavilions treated with zinc oxide at the two concentrations, hyperkeratinization and thickening of the epidermis were observed, accompanied by phenomena of desquamation. The intensity of the aforementioned degenerative phenomena was lower in the case of skin protection with zinc oxide ointment compared to the control. Additionally, with zinc oxide, the best protection was observed with the ointment containing the highest concentration, namely 13.92 mg/g lanolin (Fig. 2.c, d).

Following microscopic examinations of sections taken through the auricular pavilions treated with magnetic ointment based on lanolin, at concentrations of 6G and, respectively, 12G, cytohistological aspects observed were a relatively normal appearance, with rare vacuolated keratinocytes of epidermis, the thickness of the epidermis remaining within the normal standard.

Also, penetration of nanometric magnetic particles through the epidermis into the superficial layer of the dermis was observed, associated with endocytosis of magnetic nanoparticles by fibroblasts, macrophages, and the epithelial cells, the last ones with a known capacity for endocytosis, and which became a kind of "*pigment cells*" with a great protecting role against the UV radiation (Fig. 2e), but with no marked oedema or vascular ectasias, particular to the erythematous phenomenon, were observed (Fig. 2f).

Between the two concentrations of magnetic nanoparticles used, 6G and 12G, respectively, no essential differences were reported regarding the protective effect of the skin under conditions of prolonged irradiation. The lower concentration of magnetic nanoparticles (6G), however, was chosen since it provided an easy application for cutaneous tissue, and it was not oncutous and hard, compared to compound with nanoparticle density exceeding 12G, while still offering the same level of protection against UV radiation.

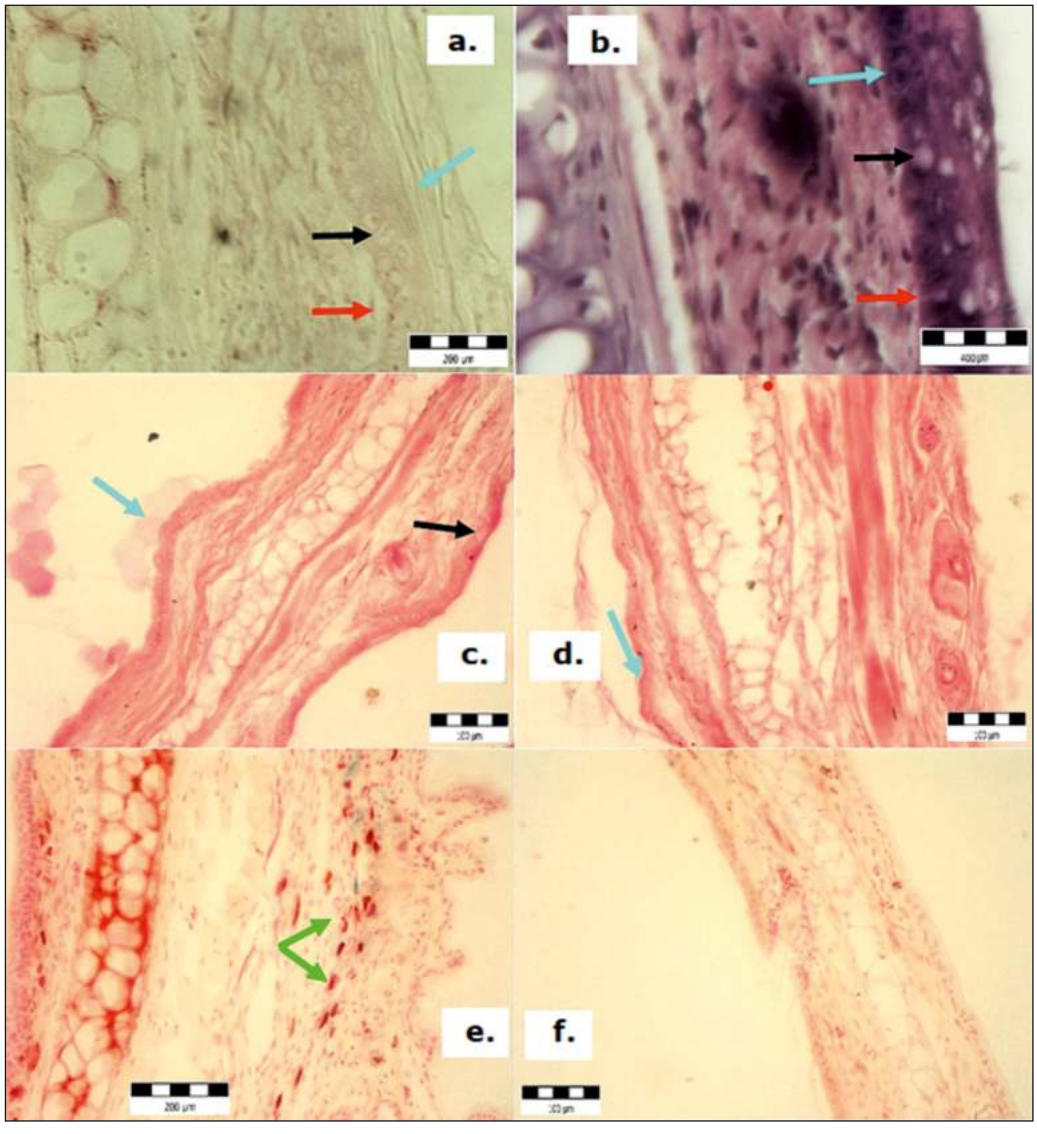

Fig. 2. Microscopic examination of sections taken through the control auricular pavilion in Experiment. Figs a and b. Section through the pinna of the control after a two-hour exposure of mice to UV radiation: hyperkeratinization and thickening of the epidermis (→); vacuolization of keratinocytes (→) and alteration of the basement membrane (→) (a. Perls staining 200 ×; b. H&E staining. 400×). Figs c and d. Section through the auricle treated with zinc oxide, in a concentration of 6.96 mg/g (c.) and 13.92 mg/g (d.) lanolin, after a two-hour exposure of mice to UV radiation: hyperkeratinization and thickening of the epidermis (→); vacuolization of keratinocytes (→) (H&E., c. 100× and d. 200×). Fig. e. Section through pinna of mice treated with 6 G magnetic ointment: endocytosis of magnetic particles by macrophages and dermal fibroblasts becoming melanocyte-like (→) (Perls, 200×). Fig. f. Section through the pinna of mice treated with magnetic ointment 12 G: normal histological appearance of the epidermis (Perls, 100×).

Experiment 2

Microscopic examination of sections taken through the control auricular pavilion in Experiment 1 is revealed in Fig. 3. During the clinical examination of the albino mice treated daily with ointment containing magnetite nanoparticles on their auricular pavilions, no changes in their behaviour were observed. Interestingly, it was noted that following daily exposure of the individuals to ultraviolet radiation for 30 minutes, pronounced erythema appeared on the control ear, but it faded or even disappeared by the next day. According to literature data, this can be explained by the absence of tyrosinase in albino animals, which consequently leads to the inability to synthesise melanin, even in the presence of a normal number of melanocytes in the epidermis (12).

Histological findings

After 7 days

During the microscopic examination of histological sections taken through the auricular pavilion protected for 7 days with the nanocomposite containing magnetite particles based on lanolin at 6G, no specific changes

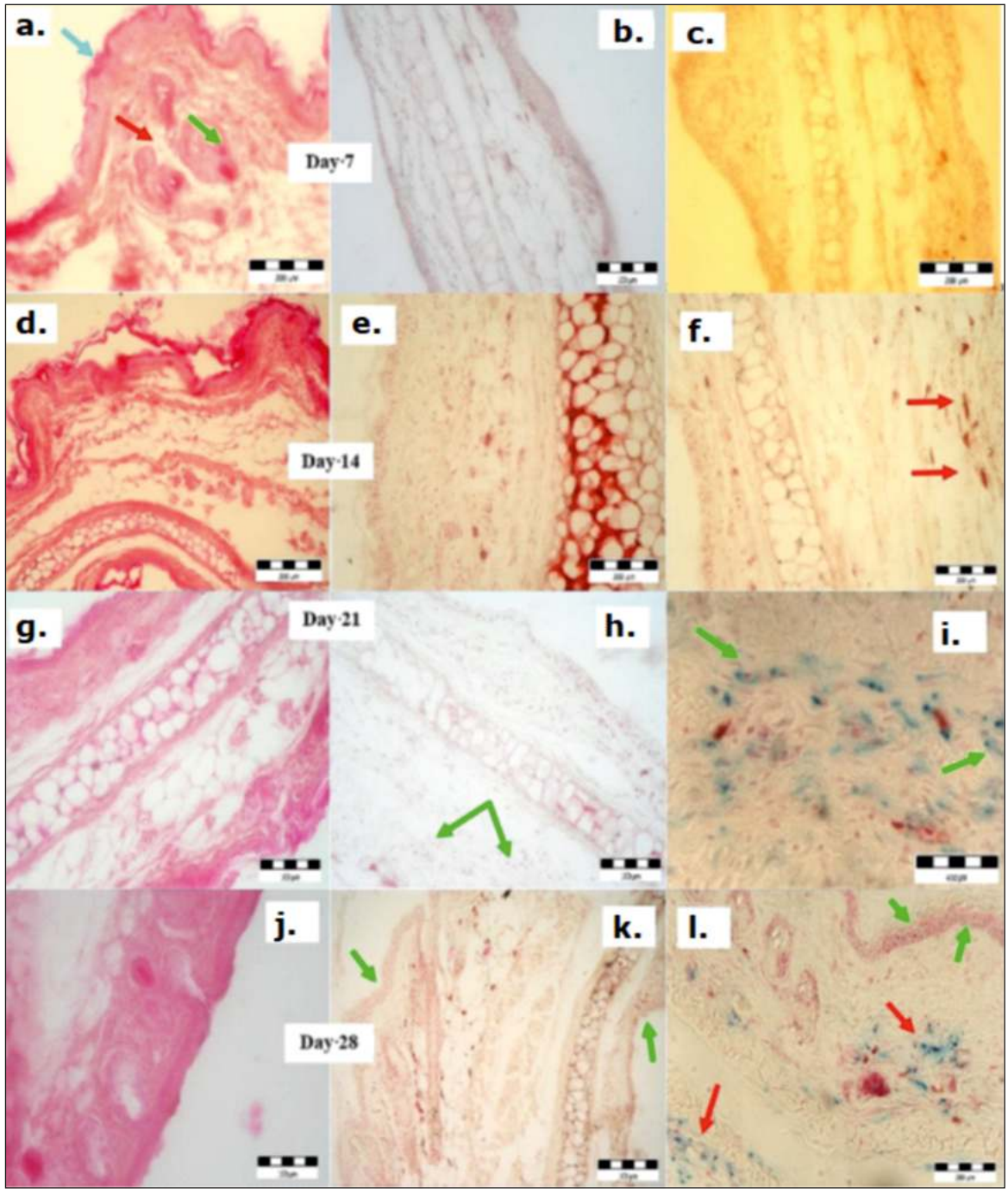

Fig. 3. Microscopic examination of sections taken through the control auricular pavilions. Figs a, b, and c. Citoarchitecture at 7 days of UV radiation; a. Control group unprotected: hyperkeratinization and thickening of the epidermis (→); degenerative phenomena at the level of the hair (→), and necrosis of the sebaceous glands (→) (H&E 200×); b. Protected with the nanocomposite containing magnetite particles based on lanolin at 6 G: normal appearance of the integument (H&E, 100×); c. Protected with the nanocomposite containing magnetite particles based on lanolin at 6 G: normal appearance of the integument (Perls, 200×). Figs d, e, and f. Citoarchitecture at 14 days of UV radiation; d. Control group unprotected: degenerative phenomena of the skin structures are more pronounced (H&E, 200×); e. Normal appearance of the integument (H&E, 200×); f. Normal appearance of the skin; endocytosis of magnetic nanoparticles by fibroblasts (→) (Perls, 200×). Figs g, h, and i. Citoarchitecture at 21 days of UV radiation; g. Control group unprotected: the persistence of the degenerative phenomena of the skin structures (H&E, 100×); h. Normal skin appearance and macrophage mobilisation (→) (H&E, 100×); i. The application of the nanocompound causes the penetration of magnetite particles in the depth of the skin and the mobilisation of macrophages (→) (Perls, 400×). Fig s j, k, and l. Citoarchitecture at 28 days of UV radiation; j. Control group unprotected: the worsening of the degenerative phenomena of the skin structures (H&E, 100×); k. Hyperkeratosis (→) (H&E, 100×); 8c. The presence of magnetic particles (→); hyperkeratosis (→); (Perls, 200×).

Fig. 4. Citoarchitecture after 14 and 21 days after exposure to UV. Figs 4a and b. Citoarchitecture after 14 and 21 days after exposure to UV - sections through the pinna of white mice protected for 7 days with G6 lanolin-based magnetite particle nanocomposite; a. Citoarchitecture after 14 days of exposure to UV: massive endocytosis of magnetic particles (→) (Perls, 200×); b. Citoarchitecture after 21 days of exposure to UV: massive endocytosis of magnetic particles (→) (Perls, 200×).

associated with exposure to ultraviolet radiation were observed compared to the control (Fig. 3a, b, c).

After 14 days

Although clinically, the erythema of the control auricular pavilions fades after each daily 30-minute exposure, histological changes in the skin of the auricular region after 14 days of exposure to ultraviolet radiation undergo increasingly pronounced degenerative processes. Histological examination of the auricular pavilions protected with the lanolin-based nanocomposite containing 6G magnetite particles for 14 days did not reveal any changes in the skin structures. In this case, the number of epidermal layers and the morphological structure of the cells did not undergo degenerative processes. In the dermis, collagen bundles and elastic fibres maintained their structure and distribution. Additionally, endocytosis of magnetic nanoparticles by fibroblasts, macrophages, and epithelial cells, cells with a high capacity for endocytosis, which become a kind of "pigment cells" with a protective role against UV radiation, was observed (Fig. 3d, e, f).

After 21 days

Cytohistological examination of the auricular pavilions protected with the lanolin-based nanocomposite containing 6G magnetite particles for 21 days did not revealed any changes in the skin structures. In this case, microscopic examination of the auricular pavilions revealed, in certain areas, infiltrations of lymphocytes and especially macrophages. We consider this phenomenon to be due to the penetration of magnetite nanoparticles deep into the dermis. These nanoparticles, being foreign bodies, are removed more slowly by tissues and cells through oxidation-reduction processes, inducing defence mechanisms in the skin, especially by mobilising phagocytes. Microscopic examination of the control auricular pavilion revealed the persistence of skin structure lesions caused by prolonged exposure to ultraviolet radiation (Fig. 3g, h, i).

After 28 days

Microscopic examination of sections taken through the auricular pavilions protected with the lanolin-based nanocomposite containing 6G magnetite particles for 28 days did not reveal the presence of significant morpho-functional changes in the skin structure. In this case, a slight hyperkeratosis of the epidermis was observed, as well as the endocytosis of magnetic particles by macrophages and fibroblasts. Microscopic examination of sections taken through the control auricular pavilions revealed the worsening of lesions resulting from prolonged exposure to ultraviolet radiation (Fig. 3j, k, l).

Making a comparison, in the case of albino mice protected with the lanolin-based nanocomposite containing 6G magnetite particles and exposed to ultraviolet radiation for 14 and 21 days, respectively, microscopic examination of the protected auricular pavilions revealed massive endocytosis of magnetic nanoparticles by fibroblasts, macrophages, and epithelial cells (cells with high endocytic capacity), which become a kind of "*melanocyte-like pigment cells*" with a protective role against UV radiation. Although small structural changes in the skin were observed, such as slight thickening of the epidermis with limited areas of exfoliation (Fig. 4).

The novelty of this research is that there was investigated what is the most appropriate dose and shape of magnetic nanoparticles, in terms of handling, adeptness, and providing a UV protective long-time effect and, on the other hand, do not cause harmful changes in the skin, especially within repeated applications, being encompassed in a series of studies, conducted in recent years, that have permitted as to observe that certain types of nano-compounds with magnetic particles can be successfully used as solar sunscreen agents for multiple administrations (11, 17, 27).

As other researchers, it was observed that there is a correlation between the size of nanoparticles and toxicity; thus, the smaller they are, the more toxic they are, probably due to the interference with the function of the cell membrane, which represents the universal, selecti-

vely permeable barrier between the cell and the external environment. At small sizes, nanoparticles enter the cell by diffusion, where they induce the synthesis of reactive oxygen species, mitochondrial damage, lysosomal dissolution,disruption of the cytoskeleton, and DNA damage (4, 34). The currently accepted optimal nanoparticle size is 50 nm, but histological analyse showed that at a size of 15 nm, nanoparticles are non-toxic to the cell. Starting from this size, the cells will resort to specific endocytosis (clathrin-caveolin dependent and/or phagocytosis) or non-specific (pinocytosis or macropinocytosis), thus cancelling the cytotoxic effect. In terms of shape, the nanoparticles with the highest internalisation rate were spherical, star-shaped, and flower-shaped (4, 34). By penetrating human or animal tissues, the nanoparticles come into contact either with serum proteins or with the connective extracellular matrix, proteins that will generate a corona, which prevents the agglomeration of the nanoparticles and reduces their toxicity, but, at the same time, can slow down the formation of reactive oxygen species, the magnetic property, etc. The genesis of reactive oxygen species represents the molecular mechanism by which nanoparticles induce cellular responses, their optimal concentration inducing tissue regeneration (4, 34).

An observed advantage of nanocompounds with magnetic particles is their stability over time, even years, during which the uniform dispersion of nanoparticles in the compounds used is maintained. This argument recommends them to be used with success in various fields as reliable UV protective screens (5, 8, 14).

A cytological aspect observed by researchers and also ascertained in the present study is the vacuolization of keratinocytes. The appearance of these vacuolar keratinocytes, identified up to the superficial layers of the epidermis, was explained by researchers as a consequence of the inability to repair DNA molecules and/or as a result of lysosome breakdown (10).

The addition of vitamin A compounds in sunscreen substances, suggested by some authors and thought to be beneficial, could be acceptable because these substances play a role in regulating and normalising the processes of skin keratinisation, maturation, and integrity of epidermal cells, ensuring the maintenance within normal limits of the multiple functions of the skin (protection, thermoregulation, secretion), but also, the role of D3 vitamin and calcium is essential, knowing that it reduces several types of UV-induced DNA damage and contributes to photoprotection, has to be evaluated when the inclusion of these molecules in nanocomposites is projected (24, 32). On the other hand, authors emphasised the risks and role of vitamin D. While sunscreen use and photoprotection can successfully reduce skin cancer risk, individuals must be aware of their vitamin D status to avoid other risks. In recent years, the role of vitamin D in calcium homeostasis and bone development has been verified, and unusual vitamin D levels have been associated with some autoimmune, neurologic, and cardiovascular diseases (4, 14).

One of the essential problems faced by the investigators has been finding dependable solutions to protect the skin from phenomena caused by prolonged exposure to solar radiation. The substances used have either a preventive or therapeutic character. Among the substances with a preventive effect are lotions, protective screens, and antioxidants - especially vitamins A, C, and E (4, 23, 37). The lotions used primarily have the effect of hydrating the skin through the evaporation of water. Well-hydrated skin is better protected against the effects of UV radiation. The most effective antioxidants for the skin have been established to be vitamins C and E, which neutralise oxygen free radicals formed under the influence of UV radiation. These free radicals induce alterations in the genetic material of skin cells and are often the cause of triggering neoplastic processes in the skin. Additionally, these free radicals act destructively on collagen and elastic fibres, reducing the skin's resistance and elasticity, which hastens the appearance of undesirable skin phenomena (25, 30, 32).

Massaging the lanolin-based nanocomposite with 6 G magnetite particles resulted in the appearance of minimal erythema in a shorter period than with light application, which influences the determination of the sun protection factor (SPF) fact observed also by other researchers. Massaging the UV protective nanocomposite leads to the penetration and dispersion of nanoparticles into the depth of the skin, reducing its screening capacity against UV radiation (15). Also, we agree with other researchers who observed that, for an effective and lasting UV protective effect, it is necessary for the nanocomposite to be applied lightly and superficially (3, 10).

In vitro tests in animal models, murine ones especially, are a good way to test ointments in dermatology. This has to be associated with the determination of the sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry (a common, simple, and rapid technique for the *in vitro* determination) (10).

CONCLUSIONS

The present study initiated a multitude of possibilities in using lanolin-based magnetic conditionings as UV protection, used as an ointment base for multifarious associations with positive skin-protecting and healing effects in humans and animals. The lanolin-based nanocomposite with magnetite particles at concentrations of 6 and 12G provides effective protection against UV radiation by applying a concentration of 0.111 and 0.222 mg nanoparticles/cm^2, respectively. The UV protective effect of the ointment used is due to the relatively uniform distribution of magnetite nanoparticles rather than their magnetisable property. The lanolin-based nanocomposite with 6G magnetite particles can constitute an effective and successful physical UV protective agent through light application. Zinc oxide, used at concentrations of 6.96 and 13.92 mg/g lanolin, exhibits a less efficient UV protective effect compared to the magnetite particle nanocomposites.

The repeated use of biocompatible nanocomposites as solar screening agents for 7, 14, 21, and 28 days doesn't induce significant modifications in the skin of animals experimentally exposed to ultraviolet radiation.

In the case of albino mice protected with the lanolin-based nanocomposite containing 6G magnetite particles and exposed to ultraviolet radiation for 14 and 21 days, respectively, microscopic examination of the protected auricular pavilions revealed massive endocytosis of magnetic nanoparticles, despite the occurrence of slender structural changes in the skin.

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Institutional Review Board Statement:

The animal study protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine from University of Life Sciences "*King Michael I*" from Timisoara (no.136/2021), For experiment the minimum acceptable number of animals was used, being in accordance with all domains' national and international relevant regulations, all methods being reported in accordance with ARRIVE guidelines. Animals were handled in accordance with directive 2010/63/EU on the handling of animals used for scientific purposes (9) and guidelines of the National Research Council (NRC) (20).

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