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Hormone treatment and UVB exposure influences on female mice regarding skin physiological parameters, biochemical parameters and organ histology

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Abstract

Females require at a certain period of life the administration or supplementation of specific hormones (estrogen, progesterone), for various needs, such as: prevention of unwanted pregnancies, decreased menstrual bleeding, dysmenorrhea and pelvic pain in endometriosis, alleviation of symptoms associated with menopause, regulation of certain skin processes related to acne or aging and others. Also, hormones could act as oncogenes being known eloquent examples of estrogens labeled both as promoters of cell specific alteration or as mutagenic agents. The use of hormones and exposure to solar radiation is expected to cause a number of adverse changes to the body, especially due to their association with malignant processes. The current study was purported as a basis for understanding certain processes that occur with the administration of hormones and exposure to ultraviolet B (UVB) radiation. The animal model was made on healthy adult female BALB/c mice, which were separated into groups and treated with Ethinylestradiol (EES), Levonorgestrel (LNG) and their combination in the presence of UVB radiation. Changes in skin physiological parameters were analyzed by non-invasive methods, biochemical parameters related to changes in blood circulating system were evaluated by standard methods and histopathological analysis was conducted to point out the changes at the level of the internal body. Measurement of skin parameters such as erythema, melanin, skin hydration, has highlighted some changes in hormone-treated and exposed to UVB radiation groups which were significant only in the case of erythema. Biochemical parameters showed variations in terms of liver enzymes in groups treated with active substances. Histologically, aspects of internal organs revealed significant changes in the group treated with EES and LNG and exposed to UVB radiation.

Keywords: Ethinylestradiol, Levonorgestrel, UVB radiation, association, parameters.

Introduction

Ethinylestradiol (EES), a chemical estrogen is the most commonly used active substance in oral hormonal formulations of contraceptive medication worldwide [1, 2]. Levonorgestrel (LNG), a synthetic progestin hormone, is commonly used in LNG-releasing intrauterine systems for its potent contraceptive effect [3, 4]. This formulation was firstly developed in 1970s, nowadays being used especially by teens because of their various advantages including decreased menstrual bleeding, dysmenorrhea, and pelvic pain in endometriosis [5]. The action of hormonal contraceptives is related to suppressing the secretion of gonadotropin-releasing hormone (from the hypothalamus) and gonadotropins (from the pituitary gland) by exerting a mechanism that prevents ovulation due to inhibition of growth of luteinizing hormone of the middle cycle and inhibition of ovarian [6].

Ultraviolet (UV) radiation, known as a carcinogen, has harmful effects on the human body that are not necessarily immediately activated and may require additional exposure to certain promoters' agents. Examples of such promoters' agents are hormones but also oncogenes, with the mention that estrogen hormones are marked mutagenic agents with direct influence on deoxyribonucleic acid (DNA) but likewise promoter agents with direct influence on cell alteration in a specific manner [7]. Nevertheless, while this type of steroid hormones is recognized as carcinogens for

certain cancers, such as: breast, uterine, and prostate carcinoma, only few studies have been conducted to study the link between these and malignant skin diseases [8]. It should be taken into consideration that certain secondary risk factors, such as hormones, both endogenous and exogenous, or ultraviolet B (UVB) radiation have been foreseen to be associated with the risk of skin cancer appearance [9, 10]. Currently, the association between use of oral contraceptives, UV radiation and occurrence of malignant diseases is controversial, not fully studied and understood.

Aim

The present study is based on the theory that many women voluntarily expose themselves to potentially harmful amounts of UVB light simultaneously with the use of hormonal contraceptives. At the same time, some willful use estrogen-based products, especially since most of those with topical application are part of the over the counter (OTC) drugs category and are easy to procure. In current experimental research, authors were studied the assessment of *in vivo* effects of: (*i*) EES, (*ii*) LNG and (*iii*) mixture of EES and LNG on mice exposed for 21 days to UVB radiations with detail on physiological skin parameters assessed by non-invasive methods, clinical and hematological parameters, and histopathological assessment.

Materials and Methods

Animal model

This animal study was conducted on adult healthy female BALB/c mice. The mice were accommodated in standard conditions: room temperature 22±2ºC, humidity ~55% and artificial lighting, half day light–half day darkness. During the experiment, the mice received special pellets and water *ad libitum*. For a week before starting the experimental study, the animals were hosted and monitored in the existing environmental conditions of the special laboratory and they have been marked for individual identification. Female mice (18–22 g) have been divided in four groups (n=6 mice/group) that were subjected to UVB radiation and which were divided as follows: a control group of healthy animals (Group 1, exposed to UVB), a group treated with EES and exposed to UVB (Group 2), a group treated with LNG and exposed to UVB (Group 3), and a group treated with EES/LNG and exposed to UVB (Group 4). The mice were exposed to UVB radiations (0.2240 J at 312 nm wavelength) for three times a week for 21 days. Administration of hormones founded in oral contraceptive medication was performed by oral gavage and animal dosage was calculated according to the formula described by Reagan–Shaw *et al.* [11]. During the study period, the body weight, the skin hydration levels, erythema and melanin measurements of the mice skin have been conducted at every two days. After 21 days, the animals were sacrificed under anesthesia. Each experimental method was realized in compliance with the norms and legislation in force applicable in the case of animal protection (included in experimental studies – Directive 2010/63/EU). The research methods were approved by the institution where the experiments were performed (Ethics Committee of Victor Babeş University of Medicine and Pharmacy, Timişoara, Romania).

Non-invasive skin parameters measurements

The measurements were carried out with a specific system, MPA5 from Courage–Khazaka, Germany. Two of the parameters, namely melanin and erythema, were evaluated by a Mexameter® MX18 probe, while for the determination of skin hydration was used a Corneometer® CM 825 probe. All measurements were done by the same operator at the same moment of day. No important changes of air humidity and temperature of evaluation room were observed from one day to another.

The evolution of every mouse weight was recorded using a laboratory balance; every measurement was done in triplicate and they were measured every second day for three weeks; the weight changes and average values were used.

Serological and hematological parameters

Sampling for blood biochemistry to assess changes in organ was realized on day 21 (end of experiment). The samples were collected in different tubes, for serological analyzes in serum flush activation tubes and for hematological analyzes in potassium ethylenediaminetetraacetic acid (EDTA) coating tubes and their processing was conducted on IL ILab 650 Chemistry Analyzer and Sysmex SF 3000 Automated Hematology Analyzer, as mentioned in the literature [12]. Blood parameters measured in the current research experiment were white blood cells (WBC) count, red blood cells (RBC) count, hemoglobin (HGB), and platelets (PLT) count. Serum parameters were selected in order to evaluate hepatic and renal status: alanine transaminase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), and creatinine (CR).

Histopathological assessment

The mice were sacrificed at the end of experiment – day 21, by applying the methods specified in *American Veterinary Medical Association* (AVMA) – anesthesia with Isoflurane and cervical dislocation, both for the animals in the control group and for those in the treated groups. Samples (liver, lungs, kidneys, spleen, and heart) were collected and were processed according to standard procedures [13] – buffered formalin fixation (4%), embedded in paraffin, sectioned (Leica rotary microtome from Leica Biosystems Nussloch GmbH, Germany) and attached to microscopic slides that were stained with Hematoxylin–Eosin (HE) for diagnosis.

Statistical analysis

The results were statistically analyzed and expressed as the mean ± standard error (SE). For the comparison between groups, the one-way analysis of variance (ANOVA) test was carried out followed by Tukey's *post-hoc* test.

Compliance with ethics requirements

Authors involved state that the study involving animal for experimental procedures conformed into specific regulation; the design method applied was studied and approved by the institution where the experiments were performed (Ethics Committee of Victor Babeş University of Medicine and Pharmacy, Timişoara).

Results

All mice present very small decreases of skin hydration in this experiment (around 1 unit/week). It can be observed in the Figure 1 that the smallest modifications were obtained in the case of the mice treated with LNG (Groups 3 and 4).

Even though all mice revealed erythema increases, Figure 2 presents small differences between experimental groups. The smallest increase of erythema index was obtained in the case of mice treated with both agents (Group 4), followed by mice Group 2 (treated with EES).

Normal increases of melanin level were observed for all mice from this experiment; everyone knows that UVB exposure leads to higher levels of melanin (Figure 3). No important changes were seen in the case of those mice treated with hormones.

Laboratory animals' weight is dependent on many external parameters, as follow: their health status, the cage sizes, the food type, the water daily consume, etc. Present study reveals chaotic modifications of mice weight with descendent trends (Figure 4). These non-linear decreases are probably due the different water and food consume from one day to another. It is important to notice that all mice had a weight loss about 1 g/week and no important difference was seen between the experimental groups.

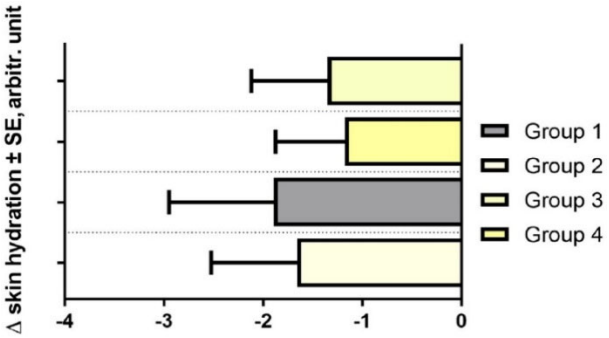

Figure 1 – ***Skin hydration evolution during the experiment. Group 1: Female mice exposed to UVB; Group 2: Female mice treated with EES and exposed to UVB; Group 3: Female mice treated with LNG and exposed to UVB; Group 4: Female mice treated with EES and LNG and exposed to UVB. EES: Ethinyl-estradiol; LNG: Levonorgestrel; SE: Standard error; UVB: Ultraviolet B.***

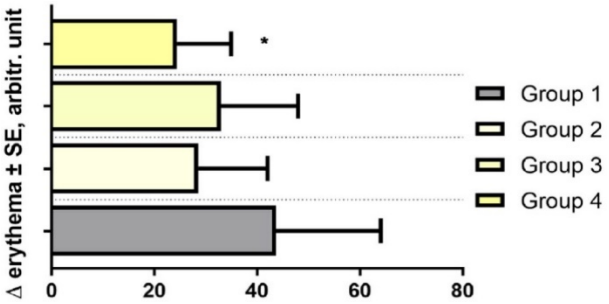

Figure 2 – ***Erythema evolution during the experiment. Group 1: Female mice exposed to UVB; Group 2: Female mice treated with EES and exposed to UVB; Group 3: Female mice treated with LNG and exposed to UVB; Group 4: Female mice treated with EES and LNG and exposed to UVB. *p<0.05 with one-way ANOVA and Tukey's multiple comparison test. ANOVA: Analysis of variance; EES: Ethinylestradiol; LNG: Levonorgestrel; SE: Standard error; UVB: Ultraviolet B.***

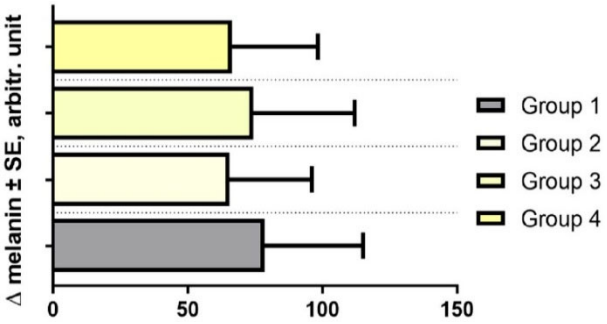

Figure 3 – ***Melanin evolution during the experiment. Group 1: Female mice exposed to UVB; Group 2: Female mice treated with EES and exposed to UVB; Group 3: Female mice treated with LNG and exposed to UVB; Group 4: Female mice treated with EES and LNG and exposed to UVB. EES: Ethinylestradiol; LNG: Levo-norgestrel; SE: Standard error; UVB: Ultraviolet B.***

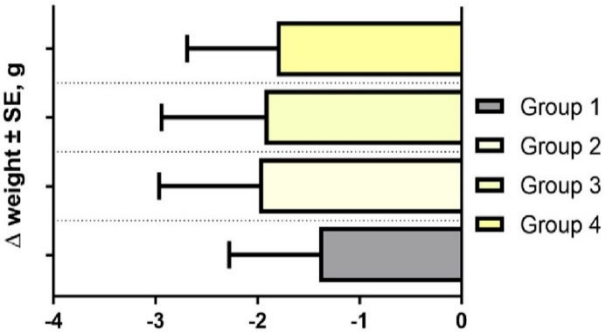

Figure 4 – ***Weight evolution during the experiment. Group 1: Female mice exposed to UVB; Group 2: Female mice treated with EES and exposed to UVB; Group 3: Female mice treated with LNG and exposed to UVB; Group 4: Female mice treated with EES and LNG and exposed to UVB. EES: Ethinyl-estradiol; LNG: Levo-norgestrel; SE: Standard error; UVB: Ultraviolet B.***

Biochemical parameters

In this UVB and hormone treatment study, no animals died in three weeks of monitoring time in all four groups. Insignificant changes or differences regarding the general appearance and weight percentage of mice were observed.

Serum parameters of mice from all groups were analyzed in order to monitor hepatic and renal health status and in Table 1 are presents the data from all groups and as it can be noticed statistically significant values, were recorded in the groups that involved the administration of hormones and exposure to UVB (observed at the end of the experiment, day 21).

Table 1 – ***Influence of UVB and association of UVB and hormones on serological parameters in mice groups at 21 days (n=3; mean ± SD)***

Time	Groups	Serological parameters			
		ALT [U/L]	*AST [U/L]*	*BUN [mg/dL]*	*CR [mg/dL]*
21 days	*1*	38±1.30	145±14.7	30.4±3.2	0.6±0.1
	2	110±36.90*	326±22.6*	22.9±1.1	0.4±0.5
	3	128±16.80*	244±48.1*	26.7±0.7	0.6±0.3
	4	146±31.50*	393±33.9*	15.8±0.3	0.9±0.7

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BUN: Blood urea nitrogen; CR: Creatinine; SD: Standard deviation; UVB: Ultraviolet B. *p<0.05 with one-way analysis of variance (ANOVA) and Tukey's multiple comparison test.

The hematological parameters showed some normal significant changes of PLT, while WBC, RBC, HGB, hematocrit (HCT) remain in the normal levels (Table 2).

Table 2 – ***Influence of UVB and association of UVB and hormones on hematological parameters in mice groups at 21 days (n=3; mean ± SD)***

Time	Groups	WBC [×10^3/μL]	RBC [×10^6/μL]	HGB [g/dL]	PLT [×10^6/μL]
21 days	*1*	11.2±1.124	8.2±0.861	10.32±1.080	349±28.45
	2	10.3±1.068	10.9±0.722	10.47±0.682	298±46.29
	3	10.7±1.505	9.1±0.586	12.51±1.115	400±17.62
	4	9.4±0.992	9.8±0.315	11.92±0.894	384±68.13

HGB: Hemoglobin; PLT: Platelet; RBC: Red blood cells; SD: Standard deviation; UVB: Ultraviolet B; WBC: White blood cells.

However, these changes may not be toxicologically significant because they have not been corroborated by biochemical results (ALT, AST). Other specific toxicity tests and several histological studies (see data related to changes in the organ level) could provide more information on the toxic effect of the compounds on the liver.

The panel of changes after 21 days of exposure was constantly different. All mice showed some changes in different organs.

In study Group 1, mice were exposed to UVB radiation, according to the described protocol. In this group, at the kidneys level, the capillary of renal corpuscles showed mild hyperemia and basophilic bodies in the lumina of some renal tubules (Figure 5a). At the lungs level, there were moderate vascular hyperemia and few inflammatory cells consisted of small lymphocytes and a mild thickening of the alveolar septa (Figure 5b). Spleen of mice exposed to UVB showed moderate red pulp hyperplasia, hemosiderin deposits in the connective tissue of the stroma and hyperemia of subcapsular vessels (Figure 5c). The changes observed in the liver consisted of mild inflammatory infiltrate at the level of portal spaces, composed of small lymphocytes, and hyperemia of centrilobular vein and venules of portal space (Figure 5d). The hearts specimens harvested from the mice of Group 1, exposed to UVB radiation, showed quasi-normal histology with traces of interstitial edema (Figure 5e).

In study Group 2, mice were exposed to UVB radiation and treated with EES. The harvested kidney specimens showed more changes compared to Group 1, hyperemia of corpuscles renal and peritubular capillaries, vacuolization of apical portion of nephrocytes and basophilic bodies in the nephrons tubular system (Figure 6a). The lungs showed thickening of the interalveolar septa, hyperemia of small vessels of interalveolar septa, increased number of peribronchial mast cells and compensatory emphysema (Figure 6b). The spleen specimens showed mild hyperplasia of red pulp and hemosiderin deposits (Figure 6c), while the liver presented mild hyperemia of centrilobular and portal spaces venules and, also, dilated sinusoidal capillaries and intracytoplasmic chromophobe vacuoles (Figure 6d). The heart specimens showed interstitial edema (Figure 6e).

In Group 3, mice were exposed to UVB radiation and treated with LNG. The kidneys showed hyperemia of renal corpuscles capillary and peritubular capillaries (Figure 7a). Lungs showed mild thickening of the interalveolar septa, hyperemia of interalveolar septa vessels and mild inflammatory infiltrate consisted of lymphocytes (Figure 7b). The spleen specimens showed mild to moderate red pulp hyperplasia, rare or few hemosiderin deposits in the connective tissue of the stroma, mild hyperemia of subcapsular vessels (Figure 7c). The liver showed mild hyperemia of centrilobular vein and vessels of portal spaces (Figure 7d). The heart specimens showed no changes of normal histology (Figure 7e).

In Group 4, mice were exposed to UVB and treated with EES and LNG. In this group were observed the most organ changes. Thus, at the level of the kidneys were observed small, atrophic renal corpuscle with mild mesangial hyperplasia, hyperemia of renal corpuscles capillaries, hyaline cylinders in some nephron tubules and acidophilic precipitates in the arterial vessels (Figure 8a). The lungs showed high thickening of the interalveolar septa, with moderate hyalinization of the arteriolar walls, mild hyperemia of small vessels of interalveolar septa. In this group, there was no lymphocytic infiltrate (Figure 8b).

Spleen showed red pulp hyperplasia, few hemosiderin deposits, mild hyperemia of subcapsular vessels (Figure 9a). Liver showed moderate hyperemia of centrilobular venules band portal vein from portal spaces, mild acidophilic precipitation of hepatocytes from acinar zone 1 and acidophilic precipitates in arterial vessels (Figure 9b). The heart presented little deviation of normal histology with mild interstitial edema (Figure 9c).

Discussions

Hormone therapy is one of the treatment options for several female diseases. One of the goals of therapy is to combat acne by counteracting the effects of androgens on the sebaceous gland by decreasing sebum production. The beautiful and healthy skin is primarily hydrated. It is pleasant to touch, supple and elastic. Environmental factors (*e.g.*, cold weather, wind) can produce excessive drying of the skin; contrariwise interior heat sources are not more beneficial, since the heating appliances drastically reduce the humidity of the air [14]. Modifications of skin hydration due to the changes of transepidermal water loss were observed in many studies of new compounds. Usually, the content of water from *stratum corneum* decrease slowly during an experiment on mice, but toxic effects can be assumed when the decrease is more than 3 units/week [15].

Erythema appears due to dilation or congestion of blood vessels. Skin color can range from reddish red till brown in patients with chronic conditions. Erythema usually results from changes in the arteries, vesicles and small blood vessels, changes that lead to increased blood infusion inside small blood vessels. Erythema can be caused by traumatization and tissue damage, changes in connective tissue and rare diseases [16]. Erythema is probably the best skin parameter used to determine any irritative effect of a chemical compound in toxicological studies. It presents small increases at every experiment on animal or human skin. UVB exposure can be considered as an important physical agent who modifies the erythema index. Alecu *et al.* described that UVB penetrates just the epidermal basal layer, rapidly causes skin redness; it stimulates the synthesis and action of vitamin D, and long exposures have carcinogenic skin effect (epitheliomas, melanomas) [17].

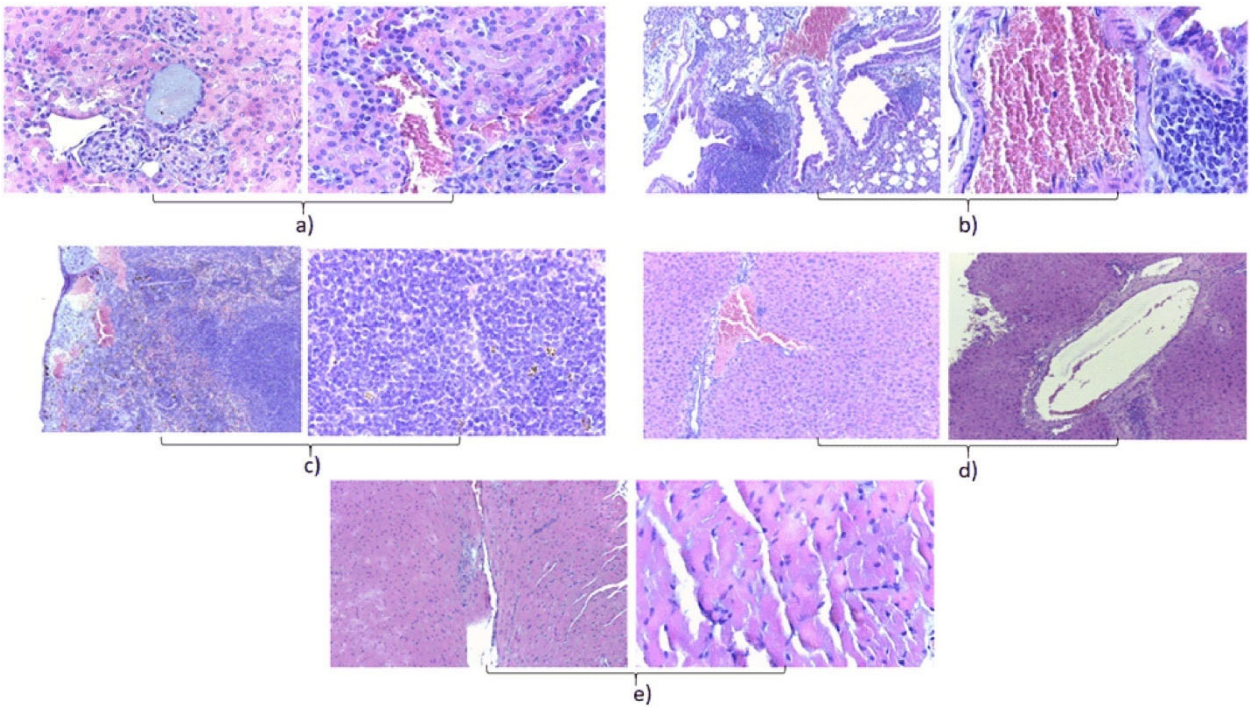

Figure 5 – ***Histological changes in female mice exposed to UVB (Group 1): (a) Kidneys – mild hyperemia of renal corpuscle capillaries and basophilic bodies in the lumina of some renal tubules; (b) Lungs – moderate vascular hyperemia and few inflammatory cells consisted of small lymphocytes and a mild thickening of the alveolar septa; (c) Spleen – moderate red pulp hyperplasia, hemosiderin deposits in the connective tissue of the stroma and hyperemia of subcapsular vessels; (d) Liver – mild inflammatory infiltrate at the level of portal spaces, composed of small lymphocytes, and hyperemia of centrilobular vein and venules of portal space; (e) Heart – quasi-normal histology with traces of interstitial edema. HE staining: ×40 (c – left, d – right); ×100 (a – left, b – left, c – right, d – left, e – left); ×200 (a – right, b – right, e – right). HE: Hematoxylin–Eosin; UVB: Ultraviolet B.***

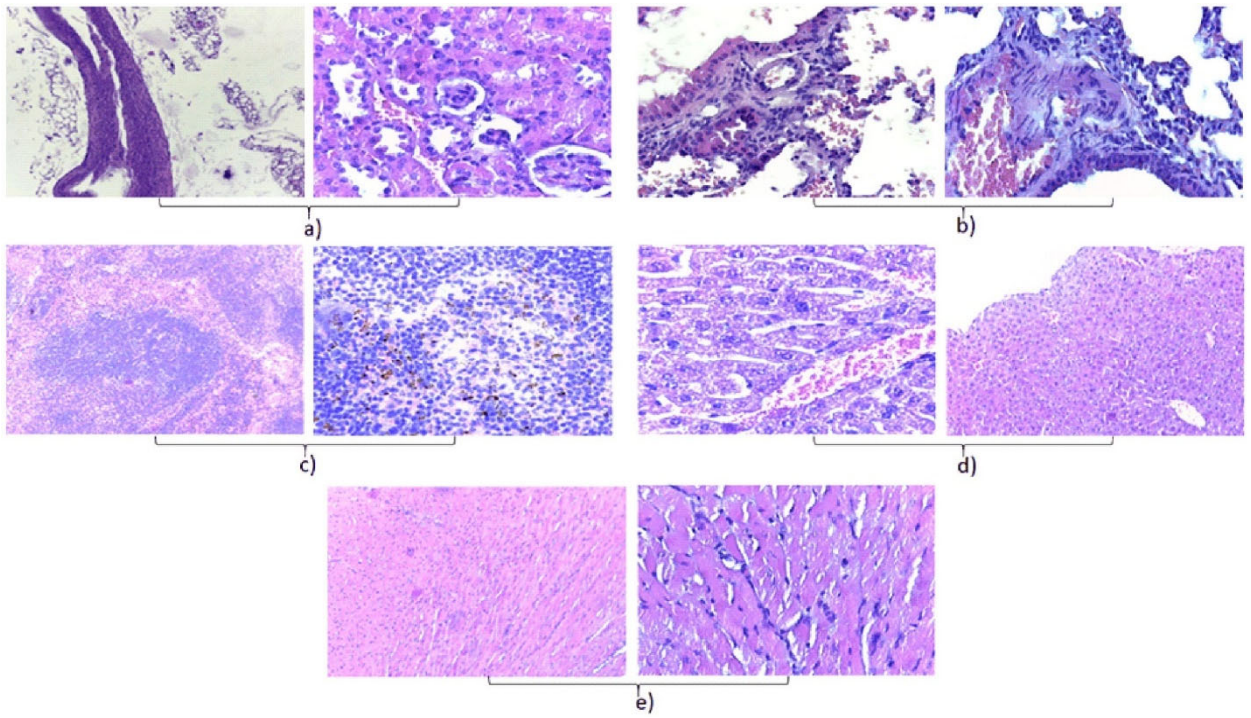

Figure 6 – ***Histological changes in female mice treated with Ethinylestradiol and exposed to UVB (Group 2): (a) Kidneys – hyperemia of renal corpuscles and peritubular capillaries, vacuolization of apical portion of nephrocytes and basophilic bodies in the nephrons tubular system; (b) Lungs – thickening of the interalveolar septa, hyperemia of small vessels of interalveolar septa, increased number of peribronchial mast cells and compensatory emphysema; (c) Spleen – mild hyperplasia of red pulp and hemosiderin deposits; (d) Liver – mild hyperemia of centrilobular and portal spaces venules and, also, dilated sinusoidal capillaries and intracytoplasmic chromophobe vacuoles; (e) Heart – interstitial edema. HE staining: ×40 (a – left, d – right); ×100 (a – right, b – left, c – left, d – left, e – left); ×200 (b – right, c – right, e – right). HE: Hematoxylin–Eosin; UVB: Ultraviolet B.***

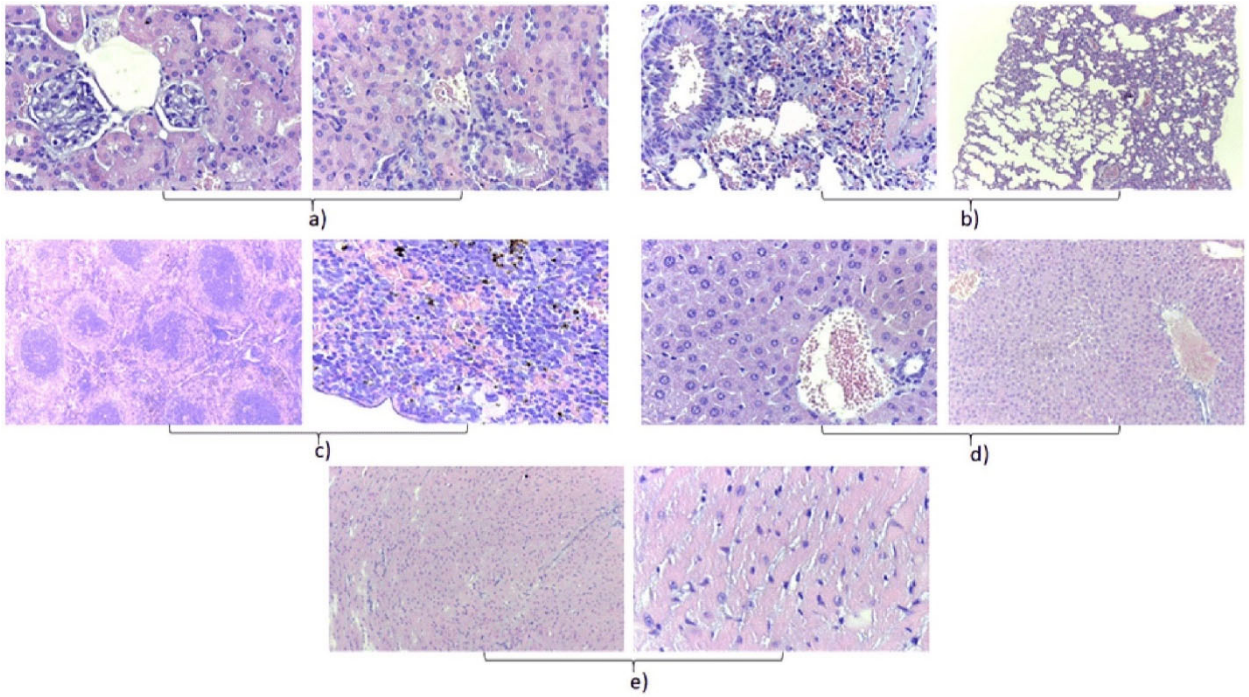

Figure 7 – ***Histological changes in female mice treated with Levonorgestrel and exposed to UVB (Group 3): (a) Kidneys – hyperemia of renal corpuscles capillary and peritubular capillaries; (b) Lungs – mild thickening of the interalveolar septa, hyperemia of interalveolar septa vessels and mild inflammatory infiltrate consisted of lymphocytes; (c) Spleen – mild to moderate red pulp hyperplasia, rare or few hemosiderin deposits in the connective tissue of the stroma, mild hyperemia of subcapsular vessels; (d) Liver – mild hyperemia of centrilobular vein and vessels of portal spaces; (e) Heart – no changes of normal histology. HE staining: ×40 (b – right); ×100 (a – right, b – left, c – right, d – right, e – left); ×200 (a – left, c – left, d – left, e – right). HE: Hematoxylin–Eosin; UVB: Ultraviolet B.***

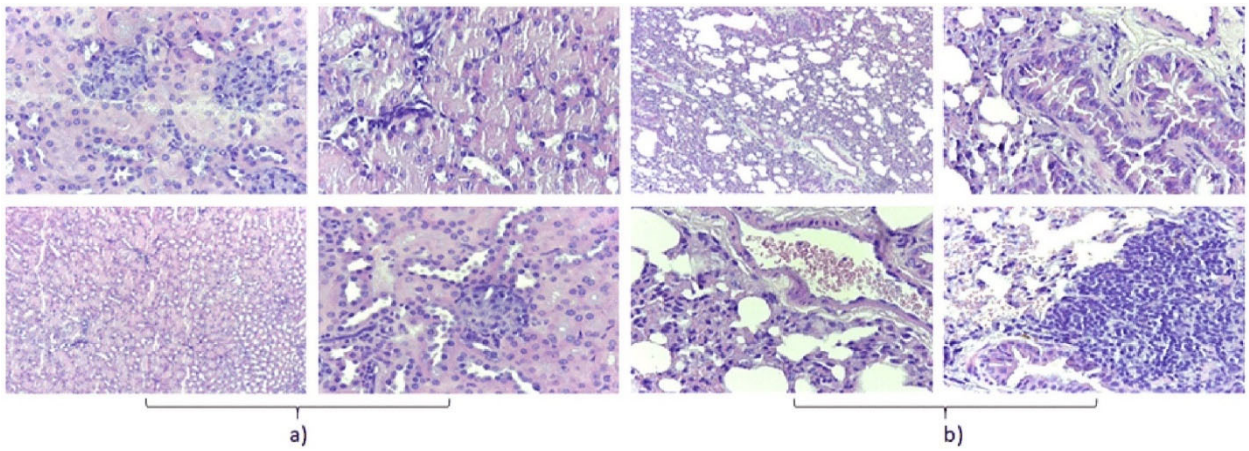

Figure 8 – ***Histological changes in female mice treated with Ethinylestradiol and Levonorgestrel and exposed to UVB (Group 4): (a) Kidneys – small, atrophic renal corpuscle with mild mesangial hyperplasia, hyperemia of renal corpuscle capillaries, hyaline cylinders in some nephron tubules and acidophilic precipitates in the arterial vessels; (b) Lungs – high thickening of the interalveolar septa, with moderate hyalinization of the arteriolar walls, mild hyperemia of small vessels of interalveolar septa. HE staining: ×40 (a – lower left, b – upper left); ×100 (a – upper left, a – upper right, a – lower right, b – upper right, b – lower left, b – lower right). HE: Hematoxylin–Eosin; UVB: Ultraviolet B.***

The group of related molecules that act in the biological system including pigmentation processes (skin, hair, eyes) is known as melanin [18]. UV radiation influences melanin differently: UVA is a mediator/catalyst of photo-oxidation processes and UVB is a stimulator/catalyst of synthesis processes through the stimulating action on melanocytes [19].

The approach to pathology through hormones is also selective and applicable to people who do not respond to standard therapies, being suitable for patients with late-onset acne, premenstrual pain, irregular menstruation or seborrhea [20].

The mechanism of action involved provides for the suppression of gonadotropin release, and implicitly of the ovarian and adrenal androgen precursors, with the increase of sexual globulin (responsible for hormone binding), correlated with the decrease of the level of biologically active testosterone. Different surveys highlighted that certain parameters (*e.g.*, biochemical, histometric) are useful in demonstrating the action of medicinal compounds on epidermal transformation regarding its metabolism and histology [21]. Currently, there is a lack of data on the impact of active drug molecules from the contraceptive

class on homeostasis of the skin and function of the sebaceous gland. The lack of an important difference between the body weight of the mice included in the current study non-treated and treated with hormones and exposed to UVB radiation may be related to a possible weight loss effect produced by estradiol, offset by fluid retention manifested by weight gain after UVB exposure and/or LNG treatment. Estradiol may have an influence on body weight by inducing weight loss due to increased basal metabolic rate unlike other drugs (such as antidepressants) that induce weight gain through also by metabolic action [22, 23]. Estrogen supplementation is associated with the prevention of skin aging (increased skin thickness and moisture, increased collagen protein levels) while decreased estrogen is associated with skin aging (dry skin, fine wrinkles, atrophy) [24]. Therefore, an increased thickness of the dermis of treated animals is most likely related to increased skin collagen due to the estrogenic compound, which also involves an increase in mitotic activity of the basal layer by stimulating the proliferation of keratinocytes [estrogen receptor alpha (ERα)] [25].

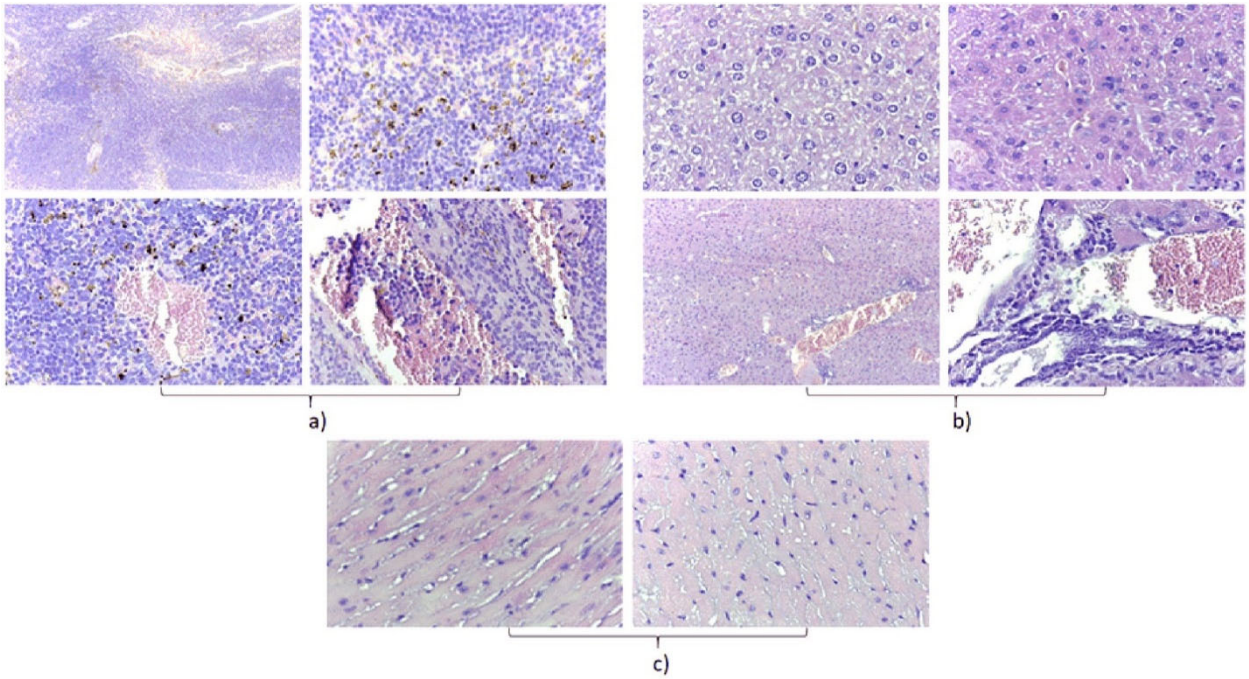

Figure 9 – ***Histological changes in female mice treated with Ethinylestradiol and Levonorgestrel and exposed to UVB (Group 4): (a) Spleen – red pulp hyperplasia, few hemosiderin deposits, mild hyperemia of subcapsular vessels; (b) Liver – moderate hyperemia of centrilobular venules band portal vein from portal spaces, mild acidophilic precipitation of hepatocytes from acinar zone 1 and acidophilic precipitates in arterial vessels; (c) Heart – little deviation of normal histology with mild interstitial edema. HE staining: HE staining: ×40 (a – upper left, b – lower left); ×100 (a – lower left, a – lower right, b – lower right, c – right); ×200 (a – upper right, b – upper left, b – upper right, c – left). HE: Hematoxylin–Eosin; UVB: Ultraviolet B.***

Skin aging is due to the action of intrinsic and extrinsic factors that result in the typology of intrinsic and/or extrinsic aging. The first typology, the intrinsic one is initiated by physiological changes, correlated with low estrogen levels with age, at genetically determined rates [26]. This process is stimulated after menopause but can be slowed down with the adoption of an estrogenic treatment that leads to positive effects, such as: increased humidity, attenuation of wrinkles, improved repair processes [27]. Extrinsic aging is initiated by exposure to factors such as UV radiation and mainly affects the parts of the body exposed to the sun (face, neck, hands). UVB is a key factor that significantly affects dermal fibroblasts even though a tiny percentage (approximately 5%) reaches the upper dermis. Currently, there are gaps related to the recovery processes of the skin chronically affected by UVB exposure, mainly due to the lack of information regarding the interconnection of intrinsic and extrinsic skin damage processes. The theory that the activation of the primary typology (intrinsic) pathway could repeal the mechanisms induced by the second typology (extrinsic) aging requires an in-depth study. The extracellular matrix plays an important role in all these processes, being subjected to complex changes and studies require not only the analysis of changes in the collagen matrix, being known that UVB radiation induces proteolytic cleavage of collagen fibrils [28]. The appearance of micro-lesions and the additional loss of total skin collagen are promoters of the functional deficiencies of the collagen matrix and the repetitive application of UVB radiation affects both hyaluronan and proteoglycans [29]. Adverse effects occur when hyaluronan interacts with specific genes, *e.g.*, gene 6 stimulated by tumor necrosis factor, with critical determinant consequences on cellular phenotypes [30]. The effect of metabolic processes centered on hyaluronan, along with the loss of estrogen hormones, is ambiguous, with the finding that acute UVB damage results in an increase in hyaluronan, while chronic impairment causes a decrease [31].

Estrogen after topical application improves firmness, elasticity and decreases the depth of wrinkles and pore size [25, 32]. Specific receptors are found in different systems:

ERα is found in the mammary gland, reproductive tissues, cardiovascular system, bones, and brain and estrogen receptor beta (ERβ) is found in reproductive tissues, lungs, bladder, heart, adrenal, thymus, kidneys, pituitary, hypothalamus, and skin. Both ERα and ERβ bind to estradiol with identical affinity, but their expression profiles are tissue-specific, with the specification that ERβ is more widely distributed in the skin. It should be noted that ERα activation is a major factor in cancer reproduction, which makes the selective targeting of ERβ a targeted therapy [32].

Estimation of serum biochemical parameters in treated animals showed different values compared to the control group. However, the transaminases (ALT and AST) were observed to be positive and showed a significant increase (p<0.001) in animals for Groups 2, 3 and 4 compared to the non-treated mice. Various studies pointed out that raised serum levels of liver enzymes (ALT and AST) are not directly related to liver damage. However increased levels are liable for causing inflammation, cell leakage and cell membrane damage to liver cells [33]. The principal target organ for the drug or bioactive active molecules is the liver, exposed to external substances being absorbed in the intestines and metabolized to other chemical products that may or may not be hepatotoxic to mice [34]. Therefore, the increase in liver enzymes after the administration of the compounds may be due to certain interactions that could have potentially toxic to the liver, with increasing dose and the result of liver damage.

Conclusions

The present study represents a first step in the analysis of the physiological parameters of the skin correlated with the modification of the biological parameters and the events that take place at organ level with the exposure to UVB radiation and the administration of active substances from the class of hormonal contraceptives. The association between the two hormones, EES and LNG, in the presence of UVB radiation has been shown to affect both skin and biochemical parameters and has led to specific changes in the organ. Estrogen being a promoter can stimulate cell proliferation with negative effects on the cell cycle with consequences of erroneous replication. Future studies are needed to investigate the mechanisms involved in the presence of UVA radiation but also a variation in age with an emphasis on specific biomarkers that correlate with the possible occurrence of malignant processes.

Conflict of interests

The authors declare that they have no conflict of interests.

Authors' contribution

Virgiliu Bogdan Şorop and Veronica Mădălina Borugă equally contributed to the manuscript.

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