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Skin condition of mice under impact of anthropogenic factors

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ABSTRACT

The toxic impact of anthropogenic factors in macroorganism can initiate autoimmune processes, involving dermis, which can cause early manifestations of genodermatoses. This factor in the agricultural region of Russia needs to be studied. Genetic mutations, congenital malformations of intrauterine growth and high dermatosis morbidity can be caused by environmental mutagens.

Objective. To study the impact of anthropogenic factors on the skin of mice and their genotoxity in the first offspring.

Material and methods. The experimental SHK outbred mice (*n*=84) were divided into 6 cages. Each cage included 10 females and 4 males. The exposure of pesticides and electromagnetic telephone's radiation were studied.

Results. In the examination of animals, dynamics of such indicators as average body weight, hair care, activity, intoxication symptoms and clinical pattern were observed, and the skin detmatoscopy of experimental series of mice and their first offspring was conducted. In the «Demitan» series the microcirculatory dysfunction in the auricle's skin and in all experimental mice (except control), namely disruption of foot skin integrity in the form of erosion, rhagades and scales were established. Hyperemia and secondary efflorescences were present in the back skin of newborns from test series on the 2nd day of life, and erosion, crusts and voluminous desquamation appear by the 5th-6th week of life.

Conclusion. The obtained results suggest embryotoxic impact of pesticides on the foetus of mice, manifested in the postnatal period by skin pathology, developmental delay and death in the first month of life. Disruptions of corneal layer integrity have been observed in adults with endogenous impact of anthropogenic factors in the first 1—2 months.

Keywords: *pesticides, mice, experiment, dermatoscopy, skin.*

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Introduction

Manifestation of epidermolysis bullosa is recorded in the first year of life in 97% of patients, and at preschool age in others in the Republic of Dagestan [1]. Toxic effects of anthropogenic factors can trigger autoimmune processes in macroorganism, involving dermis [2—6], which can cause early manifestation of genodermatoses. This factor in agricultural region of Russia should be studied.

Patients' population of the region includes simplex, junctional and dystrophic types. Closely related marriage is not a prevalent etiopathogenetic factor in population of EB in the region. Thus, according to [7], the parents of 46 (54.76%) among 84 patients with simplex EB do not have sibling relationships, and 18 (50%) among 36 patients suffer from dystrophic type of EB. A mutation associated with EB occurs in every 227th individual according to the data of foreign researchers. The genealogy study of patients with EB shows the high frequency of genetic defect among population of the Republic of Dagestan manifesting by various phenotypes of this genodermatosis [7—9]. Genetic mutations, congenital malformations of intrauterine growth, high dermatosis morbidity can be caused by environmental mutagens [10]. The number of children with congenital malformations in environmentally unfriendly regions is equal 95% [11—17]. There is a high cytogenetic mutagenic effect of environmental chemicals (pesticides, drugs, etc.) [18]. At the same time, domestic and foreign researchers established, that anomalies in the chicken and quail embryos, leading to their death, and in the offspring of rats and mice – to the formation of pathologies of kidney, brain cells, heart, liver, nervous and endocrine systems are developed under the influence of mobile phone's electromagnetic emission [19—27].

Mice and rats are the golden standard in the study of mutagenic ability of chemicals to induce DNA damage, as such experiment is considered to be phylogenetically close to the human body [18].

The reason of the high proportion of genodermatoses among the population of the Republic of Dagestan requires investigation.

Objective. To study the impact of anthropogenic factors on mice skin and their genotoxicity in the first offspring.

Material and methods.

The experimental mice of SHK outbred line (n=84) were divided into 6 cages. Each cage contained 10 females and 4 males. The effect of «Demitan» pesticide, «Titul-390» fungicide, «Ammonium nitrate» (AN) mineral fertilize, electromagnetic emission (EME), as well as their combination (combined seed) on skin and offspring of gnawing animals was examined. The seed was injected with drinking water. The animals of control group did not receive the seed. The animals and their offspring exposed to physical chemical factors during the first 2 months were examined. Study methods of animals were following: weighing, dermatoscopy, examination, photofixation.

Results and discussion.

The impact of anthropogenic factors on the skin of mice and their offspring has been studied. The dynamics of indicators, namely average weight, wool care, activity, intoxication symptoms, clinical picture **(Tab. 1**.) were registered during the examination of animals.

As seen from the **Tab. 1**, visible skin rushes in the experimental animals were not detected during the observation period. Their activity changed from high to medium by the 15th day of observation. The activity decreased to medium only by the 30th day in the EME group, and to low in the CS group. In the control group the activity remained stable high. Therefore, all studied seeds decrease motor activity of mice, and intoxication symptoms (fatigue) were noted by 30th day while using CS. Nevertheless, coat care remained at the appropriate level in all groups of mice – hair was clean and shiny.

There was a significant difference in the number of newborn mice offspring in the control and study groups according to the **Tab. 1**. Thus, 6 females brought offspring in the control group during 1 month of observation, and by 1 offspring was noted in the «Demitan», «Titul» and CS series. This indicates a negative impact of above mentioned chemicals on mice reproduction. Less than in other groups, but relatively high number of offspring was in the AN and EME series — 4 and 5, respectively. Changes in weight also vary significantly in the study and control series of animals. The latter showed an average body weight gain from 26.6 to 36 gr during month, that is the highest indicator. There was also body weight gain from 26.6 to 32 and 26.7 gr, respectively, in the AN and «Demitan» series, that is less than in control group. A decrease in average body weight was not-

Table 1. **State of observed mice series**

Series	Examined criteria													
	body weight, gr.			wool condition			number of offspring	intoxication symptoms			general condition/activity			skin pathology		
	the 1st day	the 15th day	the 30th day	the 1st day	the 15th day	the 30th day		the 1st day	the 15th day	the 30th day	the 1st day	the 15th day	the 30th day	the 1st day	the 15th day	the 30th day
Control	26.6	—	36	Clean/shiny	Clean/shiny	Clean/shiny	6	No	No	No	Good/high	Good/high	Good/high	No	No	No
Demitan	26.6	—	27.6	Clean/shiny	Clean/shiny	Clean/shiny	1	No	No	No	Good/high	Good/medium	Good/medium	No	No	No
Titul	26.6	—	26.2	Clean/shiny	Clean/shiny	Clean/shiny	1	No	No	No	Good/high	Good/medium	Good/medium	No	No	No
Ammonium nitrate	26.6	—	32	Clean/shiny	Clean/shiny	Clean/shiny	4	No	No	No	Good/high	Good/high	Good/high	No	No	No
Electromagnetic emission	26.6	—	24.5	Clean/shiny	Clean/shiny	Clean/shiny	5	No	No	No	Good/high	Good/high	Good/medium	No	No	No
Combined seed	26.6	—	26.3	Clean/shiny	Clean/shiny	Clean/shiny	1	No	No	Yes	Good/high	Good/medium	Good/low	No	No	No

Table 2. **Results of mice skin dermatoscopy**

Series	Examined body part							
	skin of the hind limb sole		skin of ear		tail hair		claws	
	the 1st day	the 30th day	the 1st day	the 30th day	the 1st day	the 30th day	the 1st day	the 30th day
Control group	Color is pink with silver gloss. There are places with accumulation of corneal masses. Microrelief of the skin – furrows, diamonds, hyperkeratosis in some places	Color is pink with silver gloss. There are places with accumulation of corneal masses. Microrelief of the skin – furrows, diamonds, hyperkeratosis in some places	Color is pink, vessels are dendritic and translucent, rare hair	Color is pink, vessels are dendritic and translucent, rare hair	Hair is whitish. Skin is translucent, pink	Hair is whitish. Skin is translucent, pink	Claws are curved, dark at the distal end	Claws are curved, dark at the distal end
Demitan	Color is pink with silver luster. There are places with accumulation of corneal masses. Microrelief of the skin – furrows, diamonds, hyperkeratosis in some places	Color is pink-silver, there are desquamation and erosions in some places, cracks in folds	Color is pink, vessels are dendritic and translucent, rare hair	Color is pink, vessels are translucent by linear intermittent petechiae	Hair is whitish. Skin is translucent, pink	Hair is whitish. Skin is translucent, pink	Claws are curved, dark at the distal end	Claws are curved, dark at the distal end
Titul	Color is pink with silver luster. There are places with accumulation of corneal masses. Microrelief of the skin – furrows, diamonds, hyperkeratosis in some places	Color is pink-silver with luster, there are desquamation and single cracks in interdigital web	Color is pink, vessels are dendritic and translucent, rare hair	Color is pink, vessels are dendritic and translucent, rare hair	Hair is whitish. Skin is translucent, pink	Hair is whitish. Skin is translucent, pink	Claws are curved, dark at the distal end	Claws are curved, dark at the distal end
Ammonium nitrate	Color is pink with silver luster. There are places with accumulation of corneal masses. Microrelief of the skin – furrows, diamonds, hyperkeratosis in some places	Color is dirty pink with dull luster, dirty yellow squama around finger pads and sole diamonds. There are erosions at the base of the nail plates	Color is pink, vessels are dendritic and translucent, rare hair	Color is pink, vessels are dendritic and translucent, rare hair	Hair is whitish. Skin is translucent, pink	Hair is whitish. Skin is translucent, pink	Claws are curved, dark at the distal end	Claws with a dirty gray stain
Electromagnetic emission	Color is pink with silver luster. There are places with accumulation of corneal masses. Microrelief of the skin – furrows, diamonds, hyperkeratosis in some places	Color is silver-pink with luster, there are cracks with hemorrhagic crusts, large-flake desquamation in some places	Color is pink, vessels are dendritic and translucent, rare hair	Color is pink, vessels are dendritic and translucent, rare hair	Hair is whitish. Skin is translucent, pink	Hair is whitish. Skin is translucent, pink	Claws are curved, dark at the distal end	Claws are curved, dark at the distal end
Combined seed	Color is pink with silver luster. There are places with accumulation of corneal masses. Microrelief of the skin – furrows, diamonds, hyperkeratosis in some places	Color is dirty pink-silver with luster, there are erosive cracks on the sole with dirty yellow squama on the periphery, petechiae, cracks in interdigital webs	Color is pink, vessels are dendritic and translucent, rare hair	Color is pink, vessels are dendritic and translucent, rare hair	Hair is whitish. Skin is translucent, pink	Hair is whitish. Skin is translucent, pink	Claws are curved, dark at the distal end	Claws are curved, dark at the distal end



Fig. 1. **Control group. The foot of mouse's posterior left limb is pink with a silver gloss.**
Aggregates of corneal masses in places. Skin microrelief: footpads are prominent, grooves divide the skin into diamonds, hyperkeratosis in places, a whitish hair covering on the periphery. The claw is curved, dark at the distal end.

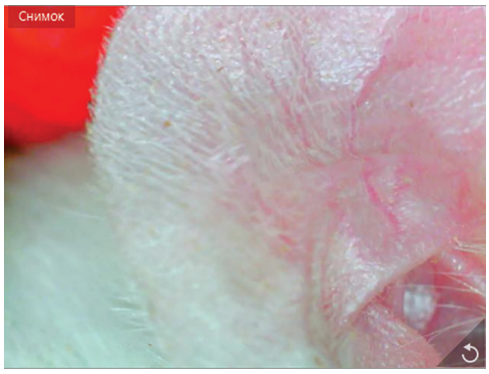


Fig. 2. **Control group. The skin of mouse's right auricle is pink, arborizing vessels are translucent, covered with rare whitish hairs.**



Fig. 3. **«Demitan» series. The skin of mouse's right auricle is pink, covered with rare whitish hairs, changes in the form of foci with interruptedly linear petechiae.**



Fig. 4. **«Combined» series. The foot of mouse's posterior left limb is dirty pink with a silver gloss.**
There is a macrolaminar desquamation, singular erosions and a rhagade with hemorrhagic crust in the second interdigital fold.

ed in other series, namely in «Titul» group — 26.2 gr, in CS — 26.3 gr and in EME – 24.5 gr.

A negative impact of exogenous factors on the studied indicators of mice has been established. Thus, «Demitan», «Titul-390» chemicals and their combination with AN and EME actually blocked the reproduction process, namely there was 6 times less offspring than in the control group. By the 30th day, CS demonstrated mild intoxication symptoms, which were represented by fatigue and low activity of the animals. The average body weight gain in group is lower than in control group or there was a decrease in average body weight in all series. This is the result of negative impact of studied environmental factors. Nevertheless, the development of visible manifestation of skin pathology in the first month was not recorded. Though, dermatoscopic examination showed pathological changes of skin in study mice **(Tab. 2)**.

Dermatoscopic changes were registered in the skin of the hind limb sole, ear, tail and claw pattern. The experimental animals were performed dermatoscopic examination in digital format in the 1st and 30th days of study. This allowed us to carry out diagnostic monitoring of changes in the studied areas of the skin. Dermatoscopic images at the beginning and on the 30th day of the study were approximately identical in the control group according to the **Tab. 2**. Secondary morphological elements indicating pathomorphological processes in the sole skin have been registered in the study series. It should be noted that the skin color of the sole is normally pink with silver luster **(Fig. 1)**.

The claws are normally curved, light with some darkening at the distal end. The skin of the ear conch is pink, has dendritic translucent vessels and is covered with rare whitish hair **(Fig. 2)**.

Similar dermatoscopic indicators in all series were found also after 1 month. The image was differed by areas of linear intermittent petechiae **(Fig. 3)**, namely a phenomenon of microcirculatory dysfunction in the skin of ear conch.

Dermatoscopic examination of the tail did not reveal any hair and skin features, but established the changes in the sole skin in all study groups, namely erosions, cracks, squama **(Fig. 4)**.



Fig. 5. **«Control» series. The foot of mouse's posterior right limb (2nd day of life) is pink with a silver gloss in the folds and on the top of crests. The claws are transparent-whitish.**



Fig. 6. **«Control series». The skin of mouse's back (2nd day of life) with expressed skin markings: diamond-shaped crests with a glossy surface, between which pink-red folds are located.**

Table 3. **Dermatoscopic features of the first generation of mice**

Series	Examined body part			
	skin of the hind limb sole		skin of the back	skin of the ear conch
	the 2nd-3th day of life	the 5th-6th week of life	the 2nd-3th day of life	the 5th-6th week of life
Control group	Color is pink with silver luster in folds and on the apex of crests. Claws are transparent-whitish.	Color is pink-silver with luster. There is a small amount of corneal masses. Microrelief of the skin – furrows, diamonds, hyperkeratosis in some places	Skin pattern is pronounced: diamond-shaped crests with a shiny surface, between which are pink-red folds	Color is pink, vessels are dendritic and translucent, rare hair
Demitan	Color is pink with silver luster. There are an erosive surface and desquamation on the metatarsus.	Color is pink-silver with dull luster, erosive areas on the lateral fingers surface at the base of the claws. There are desquamation and erosions in some places	Skin pattern is pronounced: diamond-shaped crests with a shiny surface. Single erosions and dirty grey crust on the periphery of slit-like erosion	Color is pink, vessels are dendritic and translucent, rare hair
Titul	Color is pink with silver luster. Desquamation of transparent-whitish claws	Color is pink-silver with dull luster, the skin is dry in folds (around the crests) with tightly attached yellow squama like lichenified skin. There are bloody cracks in interdigital webs	Skin pattern is pronounced: diamond-shaped crests with a shiny surface. There are areas of hyperemia with desquamation, as well as linear hyperemic stripes	Color is pink, vessels are dendritic and translucent, rare hair
Ammonium nitrate	Color is pink with silver luster. There are yellowish squama and erosions on the surface	Color is dirty pink with dull luster, there are dirty yellow squama around finger pads and sole diamonds. There are erosions at the basis of nail plates	Skin pattern: diamond-shaped crests with shiny surface. There is a desquamation. A pronounced linear hyperemia is found on the right lateral surface.	Color is pink, vessels are dendritic and translucent, rare hair
Electromagnetic emission	Color is pink with silver luster. There are multiple red erosions in the tarsus area. There is an exfoliative yellowish and transparent epithelium on the periphery of erosions	Color is pink-silver with luster, there are cracks with hemorrhagic crusts in some places and large-flake desquamation	Skin pattern: diamond-shaped crests with shiny surface and multiple punctate erosions and desquamation. One has a pink-red edematous papule	Color is pink, vessels are dendritic and translucent, rare hair
Combined seed	Color is pink with silver luster. The sole skin is covered by yellowish squama and red erosions	Color is dirty pink-silver with luster, there are erosive cracks on the soles with dirty yellow squama on the periphery, petechiae, cracks in interdigital folds	Skin pattern is pronounced: diamond-shaped crests with shiny surface. There are linear hyperemia areas with punctate brown crusts. During lateral examination – desquamation, erosions, crusts	Color is pink, vessels are dendritic and translucent, rare hair



Fig. 7. **«Demitan» series. The foot of mouse's posterior right limb (2nd day of life) is pink with silver gloss.**
There is eroded surface and desquamation on the metatarsus. The claws are transparent-whitish.



Fig. 8. **«Title» series. The foot of mouse's posterior right limb (2nd day of life) is pink with silver gloss, yellowish scales and erosions on its surface.**
The claws are transparent-whitish.

The sole skin of mice is under greater mechanical load, than other areas of the skin, and the impact of exogenous factors that caused pathological processes in it, led to increased desquamation and development of cracks and erosions.

Similar dermatoscopic examination of the sole and back skin was performed to first generation (G1) of newborn mice in all experimental series **(Tab. 3)**. The skin of newborns does not have hair in the first week, so it is convenient and informative for the above mentioned procedure. The sole of newborns is normally pink-silver with transparent-whitish claws **(Fig. 5)**.

The skin of the back is represented by luster diamond-shaped crests with pink furrows between them **(Fig. 6)**.

The changes, namely squama, erosions, crusts, hyperemia in examined body parts were revealed in the experimental series. Thus, diffused erosive surface with fine desquamation **(Fig. 7)** was found in the «Demitan» series, and large-flake desquamation with yellowish squama **(Fig. 8)** – in the «Titul» group.

There was a desquamation on the skin of the back in all groups of G1 newborns, and in some – erosions and hyperemia **(Fig. 9**, **Tab. 3)**.

In summary, we noticed manifestations on the skin of newborn mice when they were exposed intrauterine by seeds and EME. This is manifested by the enhancement of skin desquamation, its erosion and hyperemia.

Dermatoscopy of the sole skin of the same limb was again conducted in G1 animals on the 5th-6th week of life. Secondary morphological elements (erosions, cracks, crusts) were registered in all experimental series of animals except the control one **(Tab. 3)**. Dermatoscopy of the back skin was not possible to carry out again on the 5th-6th week due to the pilosis of mice, so the skin of the internal surface of ear conch, which is identical in all experimental series of mice **(see Tab. 3)**, was studied.

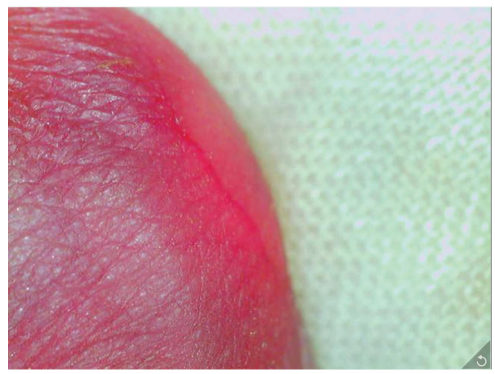


Fig. 9. **«Title» series. The skin of mouse's back (2nd day of life), skin markings: diamond-shaped crests with a glossy surface, between which pink-red folds are located.**
Desquamation is noted. There is a pronounced linear hyperemia on the right side surface.

Thus, we can conclude that under the influence of seed and EME, the skin integrity is disturbed or the desquamation of the corneal layer of epidermis is increased in the areas of the greatest traumatizing, namely the sole.

The condition of newborns and the results of their physical examination are presented in the **Tab. 4**.

The average body weight of first generation in control mice (3.08 g) was higher than that in study mice and they were highly active. The mobility criterion differed significantly in the study series. Thus, CS significantly depressed it and mobility was very low, AN and EME reduced it to low.

The Demitan impact on a pregnant organism caused superexcitation and hyperactivity in her offspring. At the same time, activity remained at a high level only in newborns in the «Titul» group, as in the control group. By the 5th-6th week, the activity of mice in all study series was noted as high, indicating that the G1 offspring has adapted to the impact of the seed and EME. The objec-

Table 4. **Objective data of the first generation of experimental animals**

Series	Body weight, gr	Wool condition by the 5th-6th of life	General condition		Skin pathology	
			the 1st day of life	the 5th-6th week of life	the 1st day of life	the 5th-6th week of life
Control	3.08	Shiny, clean	High activity	Very high activity	No	No
Demitan	1.41	Shiny, clean	Very high activity	High activity	No	No
Titul	1.8	Shiny, clean	High activity	High activity	No	No
Ammonium nitrate	1.6	Shiny, clean	Low activity	High activity	No	No
Electromagnetic emission	1.93	Shiny, clean	Low activity	High activity	No	No
Combined seed	1.4	Shiny, clean	Very low activity	High activity	No	No

tive examination did not reveal any skin pathology during the 5-6 weeks of observation of the offspring **(see Tab. 4)**.

During the development of G1 mice, significantly smaller mice than animals of their offspring were found in two study series, namely 4 in AN group and 1 in CS group. A comparative study of the above mentioned individuals has been conducted. Thus, these 4 mice in the AN-G1 study series were 3.2 times lighter than their siblings - 3.4 g versus 11.18 g, in the CS-G1 series — 3 times lighter (2.6 g versus 7.8 g) and significantly smaller in size. These mice were sedentary, ate poorly, their wool was neither thick nor shiny. Dermatoscopically, the blue tint of the paws skin and abundant yellowish scales were noted, which was not recorded in their offspring siblings. Otherwise, the dermatoscopic picture was identical. All the defective mice died at the age of 4-5 weeks.

Conclusion

The impact of the seeds and EME during 2 months has a significant influence on experimental animals. Thus, the motor activity of mice is reduced in all study series, and body weight gain and number of offspring are less than in the control group. At the same time, the microcirculatory dysfunction in the skin of the ear conch in the «Demitan» series and the violation of skin integrity of the sole in the form of erosions, cracks and scales in all experimental mice were established by dermatoscopic method.

Pathological processes have also been identified in the first generation of newborns affected by physical-chemical factors in utero. Thus, the body weight of each mouse in the offspring of the control series and their activity were higher than in the experimental animals. Hyperactivity of newborns in the «Demitan» series and high activity in the «Titul» series should be noted. Hyperemia and secondary efflorescences were present in the back skin of newborns of the experimental series on the 2nd day of life. Erosions, crusts and abundant desquamation (dermatoscopic picture) appeared on the soles by the 5th-6th week of life.

A total of 5 mice with cachexia symptoms were identified in the first generation of two experimental series (AN and CS). They were stunted in growth and body weight from their offspring siblings, they had rare wool that is not shiny enough, and there were numerous large squama on the sole. Thus, we can assume intrauterine malformations in 5 mice of experimental series, probably caused by prenatal impact of studied anthropogenic factors. These malformations are manifested on the 2nd-3rd week of life by skin and hair pathology, cachexia and cause death of animals.

The obtained results suggest embryotoxic action of pesticides on the fetus of mice, manifested in the postnatal period by skin pathology, developmental delay and death in the first month of life. Violation of corneal layer integrity are observed in adult specimen during endogenous impact of anthropogenic factors within 1-2 months.

Authors' contributions:

The concept and design of the study — M.M. Gadzhimuradova, O.V. Zhukova

Collecting and interpreting the data — M.M. Gadzhimuradova, M.N. Gadzhimuradov, M.G. Magomedov

Statistical analysis — M.M. Gadzhimuradova

Drafting the manuscript — M.M. Gadzhimuradova

Revising the manuscript — O.V. Zhukova

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