

EXPERIMENTAL ASSESSMENT OF THE EFFECT OF ANTIPSYCHOTICS BY WEDGE-SHAPED DEHYDRATION

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Recently, there has been an increase in the number of cases of unsafe use of medications, including antipsychotics, by the population. The uncontrolled use of medication leads to an increase in the rate of physical health problems and a decrease in the quality of life. Predicting the effects of substances in the body and identifying such cases are tasks of preventive medicine. The study aimed to assess the possibility to use the wedge-shaped dehydration method to determine antipsychotics (aminazine and haloperidol) in blood serum. Male outbred rats intragastrically administered aminazine and haloperidol at a dose of 35 mg/kg and 10 mg/kg, respectively, were used for the experiment. The control group was administered distilled water. Structures of blood serum facies were assessed 1, 4, 24 h after the antipsychotic administration. Parametric statistical methods were used for the analysis of comparative data ($p \leq 0.05$). Similarities of the structures of rat and human blood serum under physiological conditions were identified. The emergence of structures deviating from normal was observed in the rat facies after the haloperidol and aminazine administration. After 1 h, "silver" cracks were detected; after 4 h, rope-like structures resembling a "leaf-within-a-leaf" (following haloperidol administration) and a "double leaf" (following aminazine administration) were observed; and after 24 h, an increase in the thickness of triradiate cracks was noted. The findings demonstrated the efficacy of the wedge-shaped dehydration method in the experimental assessment of the effects of antipsychotics.

Keywords: antipsychotics, aminazine, haloperidol, wedge-shaped dehydration, experiment

Author contribution: Drugova ED — material collection and processing, manuscript writing, statistical analysis; Sheina NI — research concept and design, manuscript writing, editing; Korolik VV — research concept, manuscript editing.

Compliance with ethical standards: animal keeping and handling during the experiment were compliant with the principles of Good Laboratory Practice (GLP) and GOST 33044–2014 "Principles of Good Laboratory Practice". The study was approved by the Ethics Committee of the Pirogov Russian National Research Medical University (protocol No. 9 dated September 20, 2023. Blood collection from the volunteers, who had had submitted the informed consent, was regulated by GOST R 59778–2021 "Procedure of Venous and Capillary Blood Sampling Procedure for Laboratory Testing".

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ОЦЕНКА ВЛИЯНИЯ НЕЙРОЛЕПТИКОВ МЕТОДОМ КЛИНОВИДНОЙ ДЕГИДРАТАЦИИ В ЭКСПЕРИМЕНТЕ

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В последнее время увеличилось количество случаев небезопасного приема населением лекарственных препаратов, в том числе нейролептиков. Бесконтрольное применение лекарств приводит к увеличению нарушений физического здоровья и снижению качества жизни. Прогнозирование эффектов веществ в организме и выявление таких случаев — задачи превентивной медицины. Целью исследования было оценить возможность применения метода клиновидной дегидратации для определения нейролептиков (аминазина и галоперидола) в сыворотке крови. В эксперименте использовали беспородных крыс-самцов, которым внутривенно вводили аминазин и галоперидол в дозе 35 мг/кг и 10 мг/кг соответственно. Контрольная группа получала дистиллированную воду. Структуры фаций сыворотки крови оценивали через 1, 4, 24 ч после введения нейролептиков. Для сравнительного анализа данных использовали параметрические статистические методы ($p \leq 0,05$). Выявлено сходство структур сыворотки крови крыс и человека при физиологической норме. После введения галоперидола и аминазина в фациях крыс выявлено появление отличных от нормы структур. Через 1 ч обнаружены трещины «серебра», через 4 ч — жгутообразные структуры в виде листа в листе (после введения галоперидола) и в виде двойного листа (после введения аминазина), а через 24 ч — увеличение толщины трехлучевых трещин. Полученные результаты продемонстрировали эффективность метода клиновидной дегидратации при изучении влияния нейролептиков в эксперименте.

Ключевые слова: нейролептики, аминазин, галоперидол, клиновидная дегидратация, эксперимент

Вклад авторов: Е. Д. Другова — сбор и обработка материала, написание текста статьи, статистическая обработка данных; Н. И. Шеина — концепция и дизайн исследования, написание текста статьи, редактирование; В. В. Королик — концепция исследования, редактирование текста статьи.

Соблюдение этических стандартов: содержание животных и обращение с ними в эксперименте соответствовали правилам надлежащей лабораторной практики (GLP) и ГОСТ 33044–2014 «Принципы надлежащей лабораторной практики». Исследование одобрено этическим комитетом РНИМУ имени Н. И. Пирогова (протокол № 9 от 20 сентября 2023 г.). При отборе крови у волонтеров после подписания ими добровольного согласия руководствовались ГОСТ Р 59778–2021 «Процедуры взятия проб венозной и капиллярной крови для лабораторных исследований».

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Recently, due to changing socio-economic conditions, such as the rise of crises in global politics and the economy, as well as various stressors, and the availability of medical information online, there has been an increase in the uncontrolled use of medications, including antipsychotics, by the general population [1, 2]. It is reported that up to 60% of the entire population suffers from depressive disorders, and 91% of prescription-based pharmacy visits involve drugs that depress the central nervous system: tranquilizers, sleeping pills, antidepressants, and antipsychotics [3].

Irrational use of medications can have serious side effects and reduce the quality of life of people, therefore timely detection of the use of narcotic and antipsychotic drugs, knowledge and adherence to the principles of information hygiene in cyberspace, implementation of health measures will contribute to maintaining the population physical, mental, and social well-being (preventive medicine). There is ongoing search for predictive models for the early detection of unsafe use of such medications.

The method of wedge-shaped dehydration has become widely used in clinical practice for diagnosing pathological processes and assessing the efficacy of drug therapy. Professor V.N. Shabalin, staff members of the Department of Gerontology at the Pirogov Russian National Research Medical University, and their followers have made significant contributions to the theory and practical application of this method [4–8]. The principle of the method lies in the formation of a film (facies) upon the drying of an open blood serum droplet on a horizontal substrate. Analysis of large clinical material made it possible to identify differences in the structure of the facies of healthy people and patients with chronic and acute diseases, which provided the basis for an atlas of biological fluids in various disorders [4, 9]. It was shown that effective drug therapy led to the reduction in the number of structural markers of pathological conditions in the facies [5, 6]. Furthermore, the wedge dehydration method was recommended for the forensic medical examination of fatal poisonings involving narcotic and potent substances [10, 11].

Numerous reports present compelling evidence that the structures formed within the facies result from changes in the molecular composition and physicochemical properties of the body's biological fluids associated with various pathological conditions, and are therefore universal [12–17]. Our study is relevant as it is based on the premise that the presence of specific structures, their combinations and quantity in the facies can also represent an integral indicator of the body's overall state upon exposure to xenobiotics. Therefore, identification of changes in architectonics and structural markers characteristic of the effects of substances possessing antipsychotic properties, i.e., antipsychotics, in blood serum facies is of both scientific and practical interest.

The study aimed to assess the possibility of using wedge-shaped dehydration to determine antipsychotics (aminazine and haloperidol) in blood serum of experimental animals.

METHODS

A total of 30 male outbred rats aged 5–6 months with the body weight of 180–200 g were used in the experiment (rats were taken from the Stolbovaya breeding nursery of the Scientific Center for Biomedical Technologies of FMBA of Russia). Animals had a standard diet throughout the experiment. The rats were housed in plastic cages on a bedding of fine wood shavings. The conditions in which the animals were kept

met the requirements of GOST 33216–2014. Three groups were formed; each statistical group consisted of 10 animals.

Rats were intragastrically administered aminazine (chlorpromazine hydrochloride) at a dose of 35 mg/kg and haloperidol (a butyrophenone derivative) at a dose of 10 mg/kg—doses which, when converted to the human equivalent, corresponded to the maximum daily doses (MDD). The control group received distilled water in a volume corresponding to the volume administered to the experimental animals. Blood was collected from the sublingual vein 1, 4, and 24 h after the administration of antipsychotics; facies were prepared from the serum collected. The 20 µL biological fluid drops were applied to microscope slides using an automatic pipette. Slides were left to dry for 24 h in a room with optimal microclimatic conditions [18]. The ADF B50 digital microscope system equipped with a video camera (ADF; China) was used to view and photograph the general appearance and structures of the facies. The architectonics of the facies, the number of identical marker structures associated with pathological conditions, and the width of the zones were evaluated using the resulting images. To compare the normal facies of rats and humans, venous blood was collected from the antecubital fossa of 12 healthy volunteers, and facies were prepared using the above method.

Statistical analysis of the obtained data involved calculating the mean and standard deviation in the groups. The significance of differences between groups was determined at $p \leq 0.05$ using Student's *t*-test and Dunnett's test (q') to avoid the multiple comparisons effect. Parameters were analyzed using the BioStat software tool (Glantz; USA) [19].

RESULTS

Initially, the analysis of the facies architectonics of rat and human blood serum under physiologically normal conditions was conducted (Fig. 1).

A similarity in the facies structures of rat and human serum was noted. In both cases, the facies showed symmetry, radially diverging fractures, jointing, and concretions. The homology of facies structures corresponding to the physiologically normal conditions suggests that similar facies structures may also be found in animals and humans under certain pathological conditions and under exposure to harmful environmental factors. Furthermore, the reported similarity makes it possible to use the criteria and markers of human serum facies described in the papers by S.N. Shatokhina and V.N. Shabalin when performing morphological analysis of rat serum facies in experimental settings [4, 9].

The animals' behavior was analyzed after the aminazine and haloperidol intragastric administration. A sedative effect was observed in rats of the experimental groups one hour after the administration of aminazine and haloperidol, the presentation of which was characterized by lethargy, passivity, and reduced motor activity. The peak antipsychotic effect was observed within 4 h; no differences in clinical features between experimental and control rats were reported within 24 h.

Then the morphological analysis of the blood serum facies architecture was conducted, along with the quantitative assessment of the structures and the identification of marker structures (Table 1). One hour after the administration of aminazine and haloperidol, experimental rats showed the decrease in the width of the central zone of blood serum facies compared to the facies of the control group animals (Fig. 2, Table 1). The change was most pronounced following

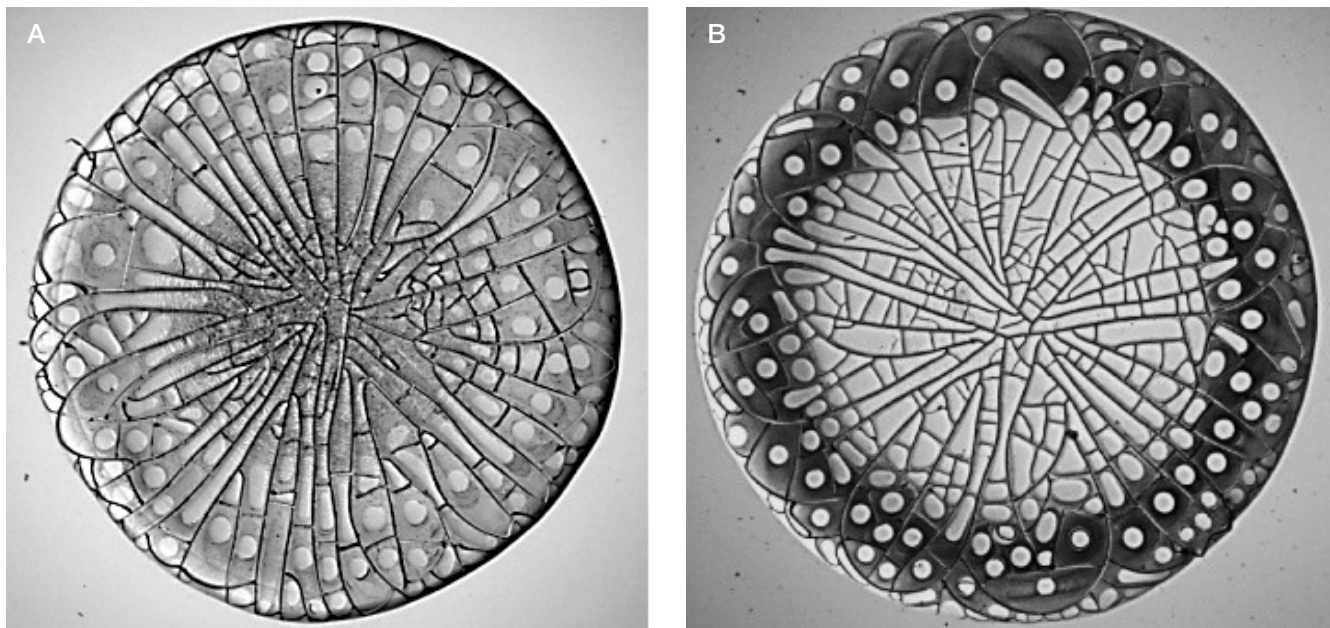


Fig. 1. Serum facies structure under physiologically normal conditions in rats and humans. **A.** Rat. **B.** Human

the aminazine administration. Assessment of the width of the facies central zone revealed a 1.3-fold decrease on average following haloperidol administration and a 2-fold decrease following aminazine administration, compared to the corresponding values of control animals.

Four hours after drug administration, leaf-like, rope-like structures were observed in the blood serum facies central zone in rats. Furthermore, rugosity was reported in this same facies zone of rat blood serum post the haloperidol administration (Fig. 3, Table 1).

Rope-like cracks were observed in the blood serum facies of rats in the experimental groups, whereas no such structures were reported in the control group animals. Furthermore, the average number of cracks of this type in blood serum facies of rats administered haloperidol was 1.9 times higher than in rats administered aminazine.

After 24 h, despite the fact that the experimental animals' behavior was back to normal and did not differ from that of the control animals, structures not found in the control group appeared in the blood serum facies of the rats administered antipsychotics. Spiral cracks were reported in the outer zone of rat blood serum facies after the haloperidol and aminazine administration (Fig. 4). The average number of spiral cracks was

significantly (1.4 times) higher in the facies of rats administered haloperidol, than in the facies of animals administered aminazine (Table 1).

It should be emphasized that such marker, as the facies surface rugosity, appeared at various times: thus, it was detected throughout the observation period after the haloperidol administration, but as late as 24 h after the aminazine administration (Table 2).

Thus, the study conducted allowed us to identify markers of antipsychotic action in rat blood serum facies, determine their quantities, and reveal similarities and differences in their effects at the levels corresponding to the antipsychotic MDDs.

DISCUSSION

The literature data suggest that the wedge-shaped dehydration method, based on fundamental processes of changes in the blood serum molecular composition and physicochemical properties is applicable in various pathological conditions and under the exposure to harmful environmental factors. In clinical trials, it is used for differential diagnosis of various diseases affecting the internal organs (of the brain, liver, ovaries, etc.), assessment of the efficacy of chemotherapy

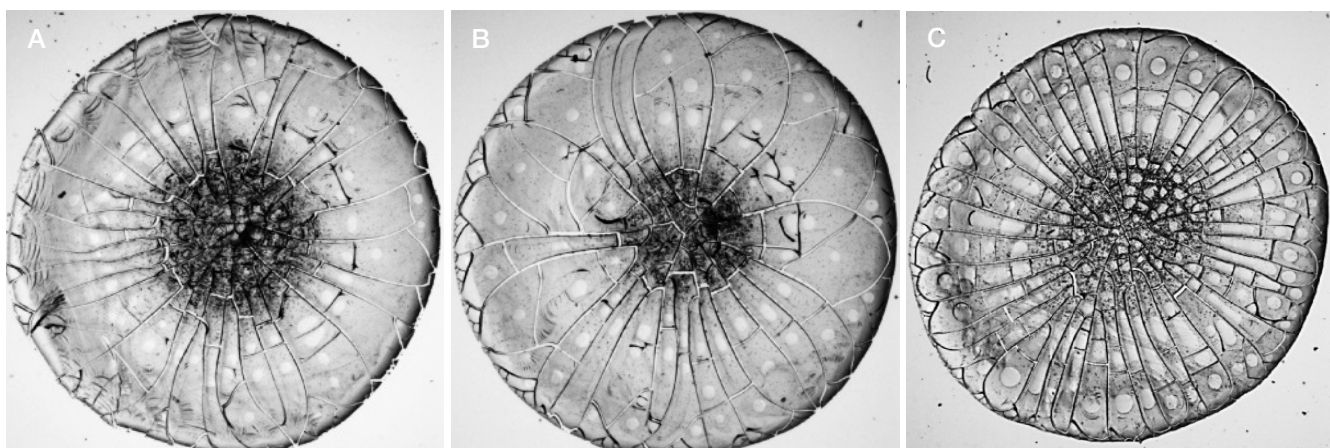


Fig. 2. Reduction in the width of the serum facies central zone in rats (general view) one hour after the administration of haloperidol (**A**) and aminazine (**B**) to rats. **C.** Control group, 12.5× magnification

Table 1. Changes in the structural markers of blood serum facies 1, 4, and 24 h after antipsychotic administration

Indicator	Groups of rats		
	Control	Haloperidol, 10 mg/kg	Aminazine, 35 mg/kg
Central zone width, % 1 h after administration	50.2 ± 4.1	37.4 ± 2.8*	24.9 ± 2.4*
Number of rope-like structures, 4 h after administration	–	5.44 ± 2.19	2.88 ± 1.71**
Number of spiral cracks, 24 h after administration	–	17.56 ± 5.01	12.50 ± 1.86**

Note: * — significant differences from the control group based on the Dunnett's test ($p \leq 0.05$); ** — significant differences from the groups of animals administered antipsychotics based on the Student's t-test ($p \leq 0.05$).

for malignant neoplasms, as well as for forensic examination of fatal poisoning by toxic substances [5–17].

The method information value is determined primarily by the early emergence of exposure markers in blood serum facies — during the period when clinical and biochemical manifestations of health problems may still be absent.

The authors aimed to compare the facies of human and animal blood serum and to attempt to substantiate an experimental model for predicting the effects of harmful substances on human health, exemplified by the effects of antipsychotics — actual substances widely used by the population as medicinal products.

There is great similarity of the general architectonics and distinct structures of the physiologically normal human and rat facies. Similarity in the architectonics of blood serum facies in humans after taking an antipsychotic and in animals after the antipsychotic administration attracts attention. Fig. 5

presents structures of the rat and young man's blood serum facies central zone 24 h after receiving/taking haloperidol: characteristic rope-like structures resembling a leaf can be seen in the blood serum facies central zone. The man had no history of chronic disorders.

The authors more thoroughly examined changes in the facies configuration and structures in the rat blood serum under the exposure antipsychotics. The study showed that the effects of aminazine and haloperidol manifest themselves in both structural similarities and differences in the facies of animal blood serum, which can probably be explained by the drug effect nature. Aminazine exerts antihistamine and anti-adrenergic effects, and a potent sedative activity. Haloperidol is a potent antipsychotic agent that blocks dopaminergic activity and possesses a moderate sedative effect [20, 21]. A sedative effect was observed in experimental rats 4 hours after administration of the drugs.

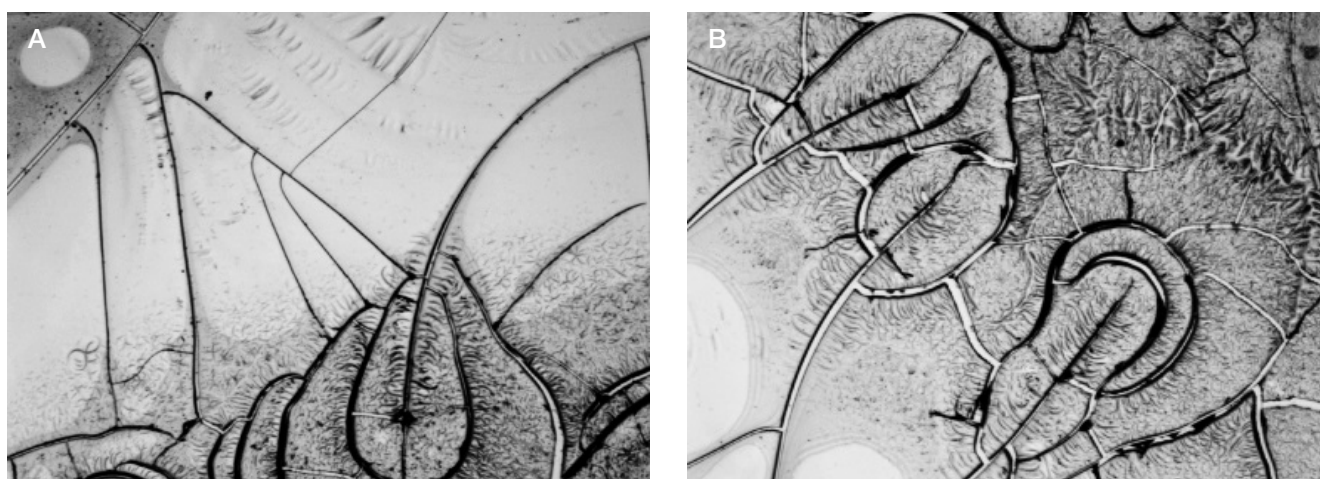
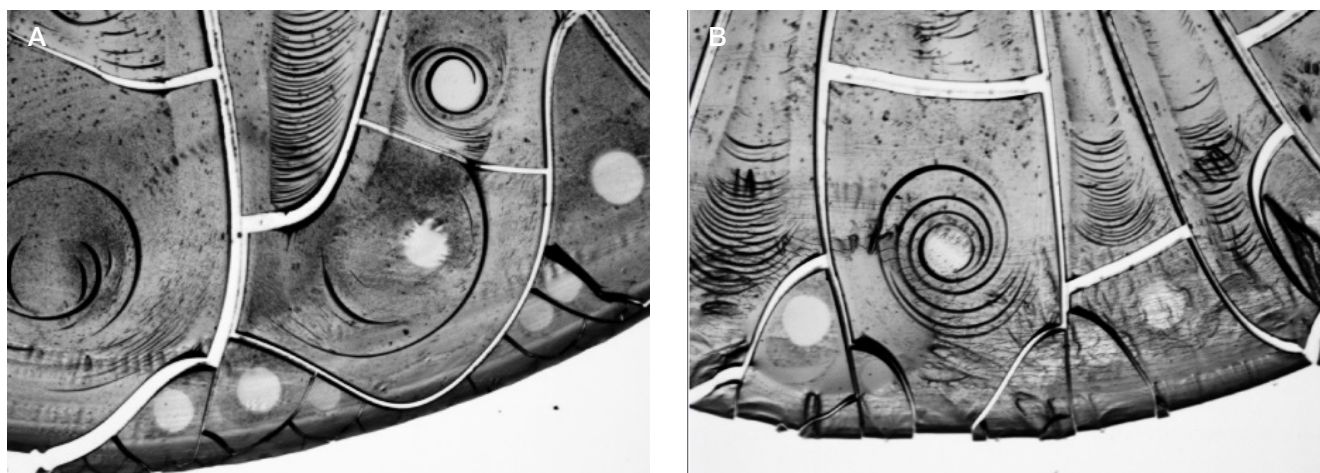
**Fig. 3.** Rope-like structures in the rat blood serum facies 4 h after the intragastric administration of haloperidol (A) and aminazine (B) to rats, 63× magnification**Fig. 4.** Spiral cracks in the rat blood serum facies 24 h after the administration of haloperidol (A) and aminazine (B) to rats, 63× magnification

Table 2. Summary table of antipsychotic markers and effects in rats at various observation time points

Antipsychotic effect	Drugs, time after administration, h								
	Haloperidol			Aminazine			Control		
	1	4	24	1	4	24	1	4	24
Behavior	+	+	—	+	+	—	—	—	—
Change in the facies general appearance	+	+	+	+	+	+	—	—	—
Rope-like structures	—	+	—	—	+	—	—	—	—
Rugosity	+	+	+	—	—	+	—	—	—
Spiral cracks	—	—	+	—	—	+	—	—	—
"Silver" cracks	+	—	—	+	—	—	—	—	—
Triradiate cracks	—	—	+	—	—	+	—	—	—

Note: + — effect; — — no effect.

The dynamic monitoring showed that "silver" cracks were found in the outer zone of the facies one hour after the administration of both drugs; the presence of those indicated impaired vascular elasticity and the blood pressure drop. These structures are presented in the atlas of human non-cellular tissues in patients with vascular abnormalities [9]. The emergence of multiple wrinkles was noted after 4 h, indicating increasing intoxication. Furthermore, after the antipsychotic administration, rope-like structures in the form of a "leaf-within-a-leaf" (after haloperidol administration) and a "double leaf" (after aminazine administration) were observed in the central zone of rat blood serum facies; the presence of those indicated hypoxia of brain cells and alterations in metabolic processes [9]. Twenty-four hours after the administration of antipsychotics, an increase in the width of triradiate cracks was observed in the central and peripheral zones; the presence of those suggested congestive phenomena in the organs. Spiral cracks were observed during this period; such cracks typically form in cases of hyperproteinemia resulting from reduced renal filtration capacity.

However, the haloperidol and aminazine effect severity and time of manifestation are different: a significantly greater number of rope-like structures and spiral cracks are reported upon administration of haloperidol; markers of intoxication appear as early as one hour after haloperidol administration and persist for 24 h. These are observed only 24 h after aminazine administration.

The authors believe that the findings and further studies will make it possible to consider the use of the wedge-shaped dehydration method in rat experiments as a prognostic

criterion for extrapolation of results to humans. This is based on the similarity of blood serum facies structures under physiologically normal conditions and the unity of mechanisms underlying the dehydration-induced self-organization of human and rat biological fluids.

In addition to the effects of antipsychotics on the animal body, the effects of other toxic substances (strychnine and arsenic oxide (V), ethanol, caffeine) possessing various biological activity and having various physicochemical properties had previously been investigated using the wedge-shaped dehydration method. It was shown that the effects of these compounds on the organism and their manifestation in the architectonics of rat blood serum facies conformed, on the one hand, to general patterns, but on the other hand, the effect had specific characteristics reflecting the biological activity of each compound [22, 23]. This confirms the scientific and practical significance of the use of this method.

It is necessary to focus on the fact that in practice, intoxication with chemical substances, including medicinal products, is determined using complex laboratory methods that require time-consuming sample preparation and expensive consumables. When considering the wedge-shaped dehydration method from an economic perspective, it is necessary to note that it has a number of advantages: it is less time-consuming allowing one to rapidly obtain preliminary results. This makes it a promising method for determining the effects of toxic substances and identifying these in preventive medicine. To ensure widespread implementation and use of the method in future, it is necessary to create a library of images of blood serum facies from experimental animals and a database of alteration post exposure to harmful chemicals.

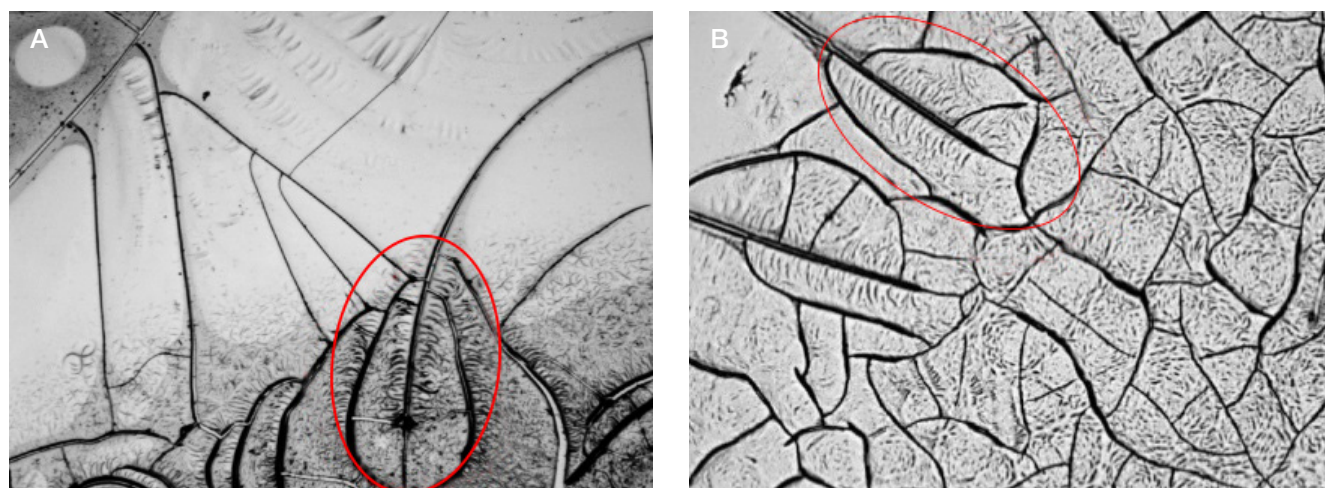


Fig. 5. Rope-like structures in blood serum facies 24 h after the haloperidol administration to the rat (A) and after the haloperidol intake in human (B) — circled in red, 63× magnification

CONCLUSIONS

Administration of antipsychotics (aminazine and haloperidol) at doses corresponding to the maximum daily doses (MDDs) caused structural changes in the dehydrated rat blood serum organization manifested as a reduction in the central zone width and the emergence of rope-like structures and spiral cracks not

found in control animals, which correlated with the experimental rats' behavioral responses. The wedge-shaped dehydration method demonstrated high sensitivity and information value in the experimental study of the antipsychotic effects. This allows us to propose it as a promising and cost-effective method for predicting the impact of chemical factors of the occupational and natural environments on humans.

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