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The Effect of Various Forms of the Drug Litholit-A on the Motor and Behavioral Activity of Experimental Animals

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Abstract **Aim** of the research was to study the effect of hydrolate from the medicinal collection on the physiological development and motor-behavioral activity of experimental animals. **Introduction.** Urolithiasis occurs in 5% of the adult population of the world and continues to grow steadily. The frequency of the disease relapses without secondary prevention reaches 80%. In spite of the expansion of the effective synthetic drugs range, herbal diuretics occupy a significant place in the treatment and prevention of this pathology. **Material and methods.** The research was conducted on 30 mongrel male rats, aged 8 weeks. The experimental animals were randomly divided into three groups of 10 each. The duration of the experiment was 30 days. To determine the specific activity of the Litholit-A herbal preparation, experiments were carried out according to the methodological manual in accordance with modern recommendations. **Results.** The medicinal herbal collection and an alcoholic tincture from the medicinal plants of the flora of Uzbekistan was produced for the treatment of urolithiasis (primary and secondary prophylactic of urinary stone formation). Both forms of the drug showed encouraging results for the metaphylaxis of stone formation in the model of calcium urolithiasis. A comparative analysis of the effect of various forms of the developed phytopreparation on the motor and behavioral activity of rats was carried out. The results presented the absence of undesirable effects in all forms of the drug. **Conclusion.** A new form of the original domestic drug Litholit-A with a 30-day administration of the drug does not significantly affect the overall development and growth of animals. Hydrolate, unlike alcohol extract, does not have an exciting effect on the psychoemotional state, but enhances the function of the gastrointestinal tract. The absence of undesirable effects of hydrolate from the collection indicates the possibility of its long-term use as a diuretic in the complex of preventive and metaphylactic measures at urolithiasis.

Keywords Herbal diuretics, Phytopreparations, Hydrolate, Experiment

1. Introduction

Urolithiasis occurs in 5% of the adult population of the world and continues to grow steadily. The frequency of the disease relapses without secondary prevention reaches 80% [1-4]. The leading causes of stone formation are functional and metabolic disorders, the correction of which requires long-term treatment. In spite of the expansion of the effective synthetic drugs range, herbal diuretics occupy a significant place in the treatment and prevention of this pathology [5-6].

Various forms of phytopreparations such as infusions, tinctures, extracts, and combined remedies are used in practical medicine. Their efficiency usually manifests itself with prolonged use. In this regard, a distinctive feature is the rarity of undesirable effects with prolonged administration [6-7].

In the process of studying the anti-lithogenic properties of representatives of the local flora, a diuretic medicinal gather from plants of the local flora was developed at the Department of Urology and Andrology of the Center for the Development of Professional Qualifications of Medical Workers. It consists of Flores millefolii, roots of Glycyrrhiza glabra and leaves of Helichrusum arenarium Samarkand [8]. An alcoholic extract was also purposefully created from this medicinal gather for the treatment of calcium urolithiasis. The diuretic properties of both the infusion and the alcoholic extract from the medicinal gather were confirmed in an experiment on mice and rats. The anti-inflammatory and anti-lithogenic abilities of the extract were revealed on the model of urolithiasis in rats (a significant decrease in the amount of deposits of calcium oxalate in the tubules of nephrons was observed). It was found that in addition to enhancing diuresis, the new preparation improved the condition of the epithelium and had nephroprotective abilities [6]. However, the presence of alcohol in the extract, in our opinion, is undesirable, which was the reason for the creation of a non-alcoholic form - hydrolate (Litholit-A). It was obtained by hydrodistillation: plants were lowered into water, brought to a boil and the resulting condensate was collected. The features of the technological process of preparing hydrolates were an indication for studying the properties and abilities of a new form of the preparation.

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Aim of the research was to study the effect of hydrolate (Litholit-A) from the medicinal gather on the physiological development and motor-behavioral activity of experimental animals.

2. Material and Methods

The research was conducted on 30 male rats, aged 8 weeks. The experimental animals were acclimatized to a room temperature of 22℃ with 12 hours of illumination and 12 hours of darkness cycles under vivarium conditions. All rats received standard nutrition throughout the course of the study and had free access to drinking water. Then they were randomly divided into three groups of 10 each:

Group I - rats were administered 1.5 ml of distilled water per os 3 times a day;

Group II - rats were administered 1.5 ml of hydrolate solution (Litholit-A) from the medicinal gather per os 3 times a day;

Group III - rats were administered 1.5 ml of 40% alcohol extract from the medicinal gather.

The duration of the experiment was 30 days. To determine the specific activity of the Litholit-A, experiments were carried out according to the methodological manual in accordance with special recommendations [9].

The physiological development and motor-behavioral activity of experimental animals were evaluated. The weight and height of the rats were measured before and at the end of the study. The tests "Open field" and "Beam-walking test" were performed to estimate the indicators of motor-behavioral activity, as well as a study of the grooming average frequency. Testing was carried out before the start of taking the drugs, on the 7th and 14th days, and at the end of the study on the 30th day.

Ethical expertise. The study was conducted in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986).

3. Results

Evaluation of the drugs effect on the physiological development of rats was studied on the basis of the weight and growth dynamics. These indicators in all groups were increased as they grew older and had no significant difference with the generally accepted criteria for normal development [10]. The difference in weight gain was observed in rats of Group III. Perhaps this is due to metabolic changes in experimental animals because of the presence of alcohol in the extract. Nevertheless, the lack of differences with the literature data indicate the safety of long-term use of the studied drugs in relation to the physiology of experimental animals (Tab. 1).

Table 1. The effect of drugs on the physiological development of rats

	weight of rats (grams)		height of rats (cm)	
Group	**Initial**	**30th day**	**Initial**	**30th day**
Group I control	215.1±3.2	319.7±3.3	18.4±1.3	19.5±1.7
Group II hydrolate	212.1±4.2	321.8±3.7	18.2±1.3	19.6±1.6
Group III extract	219.2±2.5	347.3±4.5*	19.5±1.9	21.5±1.5

Note: * - difference between groups ($p < 0.05$)

Table 2. The "Open field" test

New squares (n, M±m)				
Group	**Initial**	**7th day**	**14th day**	**30th day**
Group I control	17.20±0.68	19.20±0.68	19.80±0.83	19.44±0.84
Group II hydrolate	16.70±0.63	19.20±0.79	17.60±0.76	16.70±0.63
Group III extract	17.30±0.83	28.70±1.27	24.30±1.05	28.70±1.27
Inner squares (n, M±m)				
Group	**Initial**	**7th day**	**14th day**	**30th day**
Group I control	4.2±0.39	4.7±0.54	4.9±0.53	4.39±0.39
Group II hydrolate	3.7±0.42	4.3±0.58	3.6± 0.34	3.7±0.42
Group III extract	4.8±0.47	7.4±0.37	5.1±0.46	5.9±0.53
Total distance covered (м, M±m)				
Group	**Initial**	**7th day**	**14th day**	**30th day**
Group I control	4.28±0.19	4.78±0.22	4.94±0.24	4.77±0.21
Group II hydrolate	4.08±0.14	4.70±0.18	4.24±0.18	4.08±0.14
Group III extract	4.54±0.22	6.92±0.32	5.88±0.26	6.92±0.32
Number of fecal boluses (n, M±m)				
Group	**Initial**	**7th day**	**14th day**	**30th day**
Group I control	3.90±0.23	4.10±0.35	4.60±0.37	4.82±0.26
Group II hydrolate	5.20±0.29	6.90±0.23	7.10±0.35	8.20±0.29
Group III extract	4.60±0.31	6.30±0.42	5.60±0,31	6.30±0.42

Note: * - difference between groups ($p < 0.05$)

Determining the degree of a living organism activity is considered one of the necessary stages in experimental research. The effect of the drug on the psychoemotional state of animals was assessed by determining mobility. The behavioral reactions of rats were studied in the "Open Field" test by registering the locomotor activity of experimental rats within an hour after administration of the drug [11].

The reaction of rats which were given placebo (Group I) and hydrolate from the medicinal gather (Group II) in the "Open Field" test was characterized by a calmer state of normokinesia. There was a change in the emotional status of animals: short-term grooming prevailed, but its total duration was decreased. An increase in the number of fecal boluses 30 minutes after administration of the drug also indicated a change in the psychoemotional state of the animals, after 48 hours this indicator corresponded to the control value.

Rats who were given alcohol extract (Group III) in the "Open field" test moved in leaps, which characterizes the state of hyperkinesia. After intoxication, horizontal and vertical motor activity significantly increased in animals in compare with the control group and hydrolate. An exacerbation of motor activity was observed within 1 hour after the administration of the drug. As a result of more active movement around the installation area, the total distance traveled in Group III increased by 1.5 times, while in the control Group I and in Group II the difference was insignificant - 1.1 times ($p<0.05$). According to the number of fecal boluses, a significant increase was found in rats of Group II that received hydrolate. Apparently, here attention should be paid to the presence of a laxative effect of hydrolate (Tab. 2).

The "Beam-walking" test is used to assess the sensorimotor function of the fore and hind limbs [12]. In this test, the animal must cross the entire length of the board, which is gradually narrowed and enter the dark compartment. The placement of the paw on the upper or lower board was recorded. We counted the number of limb positions on the lower board (errors), the number of limbs slipping from the upper board to the lower one (when the brush or foot was placed on both boards) and the total number of moves made from the starting line to the animal entering the dark compartment. For the front and back paws, the calculation was carried out separately. The severity of sensorimotor deficit was calculated by the formula in percents:

$$\frac{(\text{Error} \pm 0,5 * \text{Slipping}) * 100}{\text{total number of steps}}$$

When tested on the "Narrowing path" installation, the number of hind limbs in rats of Group III already on the 7th day of the experiment significantly exceeded the same indicator in Group I (control) and Group II (30.5±1.57 vs. 15.0±1.0 and 15.6±0.98, respectively; $p<0.01$) with the same test execution time (Tab. 3).

Grooming in rats includes cleaning the coat, washing the muzzle and scratching. It makes up 15-20% in the rhythm of daily motor activity. According to the literature, usually the average frequency of grooming (once / 30 min) in stress-resistant individuals is - 19.15; with an average degree of stress-resistance - 22.75 and in stress-unstable individuals – 26. In our observations, the average frequency of grooming in all experimental animals was about 19 times in 30 minutes. But on the 7th day of observation, the average frequency of grooming in the group of animals receiving alcohol extract from the collection increased sharply and was 1.6 times higher (by 58.3%) in compare with the control and the hydrolate group. The difference remained significantly higher in the subsequent periods of the experiment (39.2% and 29.6%, respectively). The presence of an alcoholic component in the extract had a significant effect on the body of experimental animals. As a result, they become stress-resistant, which is an undesirable effect of the alcohol type of the drug. The form of hydrolate, at all times, had no significant difference with the control (Tab. 4).

Table 3. The "Beam-walking" test

The number of retreats by the hind limbs (n, M±m)				
Group	**Initial**	**7th day**	**14th day**	**30th day**
Group I control	14.5±1.17	15.0±1.0	15.2±0.95	17.5±1.17
Group II hydrolate	15.0±1.29	15.6±0.98	14.6±1.3	15.5±0.81
Group III extract	14.5±0.90	30.5±1.57	29.4±1.08	29.5±1.25
The severity of sensorimotor deficit (%, M±m)				
Group	**Initial**	**7th day**	**14th day**	**30th day**
Group I control	57.0±4.48	44.2±2.93	44.6±2.58	45.8±3.95
Group II hydrolate	52.3±4.17	44.5±2.86	40.8±2.75	45.5±2.81
Group III extract	51.9±3.21	75.1±2.81	62.8±4.61	68.3±7.13

Note: * - difference between groups ($p < 0.05$)

Table 4. Average Grooming Frequency Estimation

Group	Initial	7th day	14th day	30th day
Group I control	19.3±0.58	19.9±0.48	19.9±0.53	22.3±0.63
Group II hydrolate	19.4±1.0	20.4±1.0	18.8±0.55	21.1±0.86
Group III extract	19.2±0.61	31.5±0.93*	27.7±0.76*	28.9±0.72*

Note: * - (once / 30 min; M±m; difference between groups ($p < 0.05$))

4. Conclusions

A new form of the original domestic medicine Litholit-A with a 30-day administration, does not significantly affect the overall development and growth of animals.

Hydrolate, unlike alcohol extract, does not have an exciting effect on the psychoemotional state, but enhances the function of the gastrointestinal tract.

The absence of undesirable effects of hydrolate from the collection indicates the possibility of its long-term use as a diuretic in the complex of preventive and metaphylaxis measures at urolithiasis.

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