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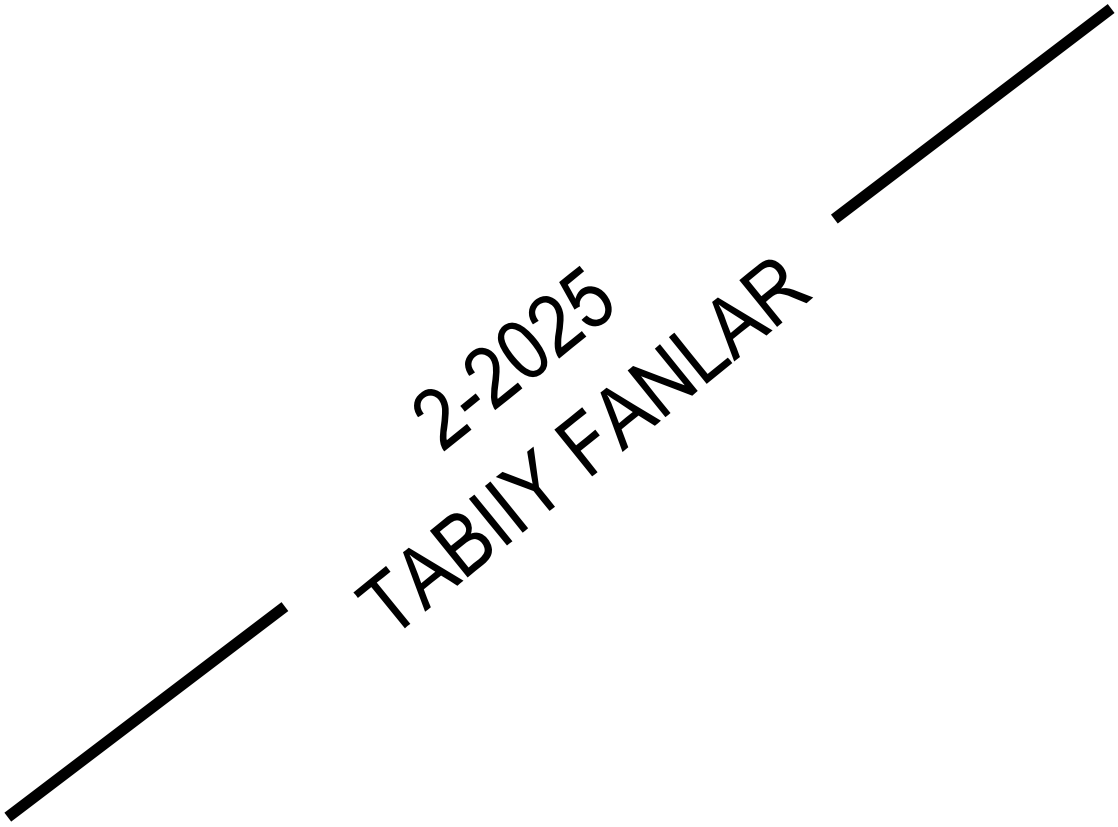

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Издаётся с 1995 года
Выходит 6 раз в год

KIMYO

BIOLOGIYA

FarDU. Ilmiy xabarlar – Scientific journal of the Fergana State University

Volume 31 Issue 2, 2025-yil DOI: 10.56292/SJFSU/vol31_iss2/a198

UO‘K: 615.015+615.89+612.39

METHODS FOR DETERMINING THE ACUTE POISONING AND CUMULATIVE PROPERTIES OF A NATURAL REMEDY “AS-SULTAN”

МЕТОДЫ ОПРЕДЕЛЕНИЯ ОСТРОГО ОТРАВЛЕНИЯ И КУМУЛЯТИВНЫХ СВОЙСТВ НАТУРАЛЬНОГО ЛЕКАРСТВЕННОГО СРЕДСТВА “АС-СУЛТАН”

“AS-SULTON” TABIIY VOSITASINING O‘TKIR ZAHARLASH VA KUMULYATIVLIK XUSUSIYATLARINI ANIQLASH USULLARI

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Abstract

Based on local plants a new biologically active food additive developed As-Sultan – a natural food supplement that replaces certain synthetic drugs that help prevent and treat gallbladder inflammation acute toxicity and Preclinical tests were conducted to determine the cumulative properties. According to the results of the experiments, it was determined that the average lethal dose of the As-Sultan food additive is LD50>10000 mg/kg. The chemical compounds were classified as belonging to the relatively harmless VI class of acute toxicity. It was proven that they do not have a cumulative property.

Аннотация

На основе местных растений была разработана новая биологически активная добавка к пище “Ас-Султан” – натуральная пищевая добавка, заменяющая некоторые синтетические препараты, которые помогают предотвращать и лечить воспаление желчного пузыря, острую токсичность, и были проведены доклинические испытания для определения кумулятивных свойств. По результатам экспериментов было определено, что средняя смертельная доза пищевой добавки “Ас-Султан” составляет LD50>10000 мг/кг. Химические соединения были отнесены к относительно безвредному VI классу острой токсичности. Было доказано, что они не обладают кумулятивным свойством.

Annotatsiya

Mahalliy o‘simliklar asosida ishlab chiqilgan yangi biologik faol oziq-ovqat qo‘shilmasi As-Sulton tarkibida o‘t pufagi yallig’lanish kasalligini oldini olish va davolashga yordam berish xususiyatiga ega bo‘lgan ayrim sintetik preparatlarni o‘rnini bosuvchi tabiiy oziq-ovqat qo‘shilmasining o‘tkir zaharliligi hamda kumulyativ xossalarini aniqlash yuzasidan klinikoldi sinovlari o‘tkazildi. Tajribalardan olingan natijalarga ko‘ra As-Sulton oziq-ovqat qo‘shilmasining o‘rtacha o‘lim dozasi LD_{50}>10000 mg/kg ekanligi aniqlandi. Kimyoviy birikmalarning nisbatan zararsiz VI sinfiga mansubligi o‘tkir zaharliligi bo‘yicha tasniflandi. Kumulyativ xossaga ega emasligi isbotlandi.

Keywords. *Acute toxicity, spasm, Student's t-test, cumulative, coefficient, “open area”.*

Ключевые слова. *Острая токсичность, спазм, t-критерий Стьюдента, кумулятивный, коэффициент, “открытая зона”.*

Kalit so‘zlar. *o‘tkir zaharliligi, spazm, Styudentning t-mezoni, kumulyativ, koeffitsiyenti, “ochiq maydon”.*

INTRODUCTION

Due to their rich chemical composition and healing properties, medicinal plants have been widely used in folk medicine since ancient times. Therefore, today it is of great importance to

develop, study the composition and properties of natural medicinal food supplements based on medicinal plants that are environmentally friendly, harmless, and economically efficient [1]. In our republic, natural food supplements that can help prevent and treat many diseases, as well as increase the immunity of the human body, are being developed and put into practice [2]. Scientific research is being conducted to create natural food supplements based on local plants that can replace some synthetic drugs that can treat and prevent gallbladder inflammation [3]. Al-Sultan - the composition of the new biologically active food supplement has been shown to contain a large amount of macro- and microelements, water-soluble vitamins and phenolic compounds that have a positive effect on the treatment of inflammatory diseases of the gallbladder, and it has been proven to have high antioxidant and antiradical activity.

In 1900, the expression of the quantitative toxicity of a substance was suggested by “Minimally lethal”, the quantitative “Moderate lethal dose” of the substance (MLD), or LD50 TREVAN. Acute toxicity studies are conducted to assess the effect of the substance on the body, not for the biological standardization of drugs [4].

Today, the following methods proposed to calculate LD_{50} are used by researchers: the Carber method, the arithmetic method Reed and Muench, the method of Litchfield and Wilcoxon, the Miller and Tainter method, the moving average method, the Lorke Method [5].

A moderate lethal dose is a dose that destroys 50% of animals when the substance is once injected into the stomach. Mean effective dose (DL_{50}) refers to the amount of substance that can cause a certain effect in 50% of the Standard Group of animals at a certain period of subsequent observation. The study of cumulative effects is considered particularly important in addressing environmental protection issues, as there are also cases where a very small amount (of substances) accumulates or concentrates in trophic (food) chains, affecting over a long period, sometimes over the life of one or more generations. Cumulative is determined by the cumulative coefficient. Acute poisoning is a disease that occurs after a single exposure of a harmful substance to the body. Poisoning that occurs can end in rapid recovery, lead to death, or, as a result, cause serious health damage.

Cumulation occurs in cases of accumulation and solid fixation of harmful substances in a certain area of the body. In this case, after exposure to a harmful substance, the distorted functions of the biological object are not fully restored, small changes accumulate, resulting in the occurrence of a pathological process [6].

This natural remedy created by us acute toxicity and preclinical tests conducted to determine the cumulative properties are presented below.

THE EXPERIENCE PART

As-Sultan food supplement acute toxicity was studied in outbred white mice of both sexes with a body weight of 18±2.0(n=18)). Experimental animals were kept in the vivarium of the Institute of Biophysics and Biochemistry under standard conditions. The animals initially underwent a 14-day quarantine period in this vivarium, after which examinations were conducted to isolate sick and injured animals. Mice were divided into groups of 6 for each experiment. The Litchfield and Wilcox on method was used to determine acute toxicity parameters [7]. As-Sultan Food Supplement In the form of a 25% aqueous solution, 5 experimental groups of animals were administered once into the stomach in a volume of no more than 0.5 ml. At doses of 4000, 5000, 6000, 8000 and 10000 mg/kg entered. Doses of more than 6000 mg/kg were administered 2 times with an interval of 1 hour. Animals of the control group were administered 0.5 ml of distilled water. On the first day of the experiment, animals were observed in laboratory conditions every hour, using viability, general condition, possible spasms and deaths as indicators of their functional state. On the remaining days, for 2 weeks, the general condition, activity, changes in behavior, respiratory rate and depth, fur and skin coverings, tail condition, amount and composition of feces, urine excretion rate, changes in body weight and other indicators were recorded daily in vivarium conditions. All experimental animals were kept under the same conditions with free access to water and food on a common diet.

As-Sultan Food Supplement in addition, the acute toxicity of rutin was studied to provide a comparative characterization. As-Sultan Food Supplement was also administered, in the same doses, but due to its low solubility, solutions at higher doses were dissolved in smaller amounts.

Initial body weight was measured on the first day of the experiment before the drug was administered and measurements were taken weekly until the end of the experiment. At the end of the experiment, the average lethal dose was determined using the generally accepted method (LD_{50}) was calculated. As-Sultan Food Supplement Classification according to acute toxicity of the Organization for Economic Cooperation and Development was carried out according to the modified rule.

Statistical analysis of the results of body weight dynamics of acute poisoning indicators was performed using Student's t-test at the r<0,05 level.

The cumulative properties of the drug were determined according to the method of Lim Et Al [8] and Al-Sultan sample The cumulation coefficient - K_k was estimated for the experiments. Body weight 18±2.0 g. wasThe study was conducted on 10 outbred white mice of 2 sexes. The drug was administered once for 28 days at the following doses: 0,1; 0,15; 0,22; 0,34; 0,50; 0,75 and 1,12 of the median lethal dose determined by LD_{50}=10000 mg/kg, increasing every four days. The sum of the doses administered during the 28 days of the experiment is 12,7 LD_{50}. The cumulation coefficient was calculated by the following formula:

$$K_k = LD_{50n} / LD_{50\text{-}1}$$

where K_k - is the cumulation coefficient;

LD_{50n} – median lethal dose after multiple administration;

$LD_{50\text{-}1}$–median lethal dose after a single injection;.

The results obtained.

Tests to determine the chronic toxicity of As-Sultan food additive

As-Sultan Food Supplement chronic poisoning characteristics of body weight of both sexes148±The study was conducted on 20 white outbred rats weighing 21 g and 3 Chinchilla rabbits weighing 2,5-3,0 kg [9]. The As-Sultan food additive was administered orally in doses of 5,0; 10,0 and 20,0 mg/kg to rats and 2,0; 4,0 and 8,0 mg/kg to rabbits for 30 days by probe. Blood samples were taken from the animals for analysis on days 10 and 30 of the experiment, and diuresis analyses were performed on the same days.

In chronic poisoning studies conducted as part of preclinical trials of the As-Sultan food supplement, its effects on peripheral blood cell composition, liver and kidney function were evaluated using cytological and biochemical methods, as well as its effects on the blood coagulation system using thromboelastography.

On days 10 and 30 of the study, the cellular composition of the peripheral blood of rats was determined using a Motic BA210E microscope for platelets, leukocytes, erythrocytes, and reticulocytes.

Changes in blood clotting in animals under the influence of As-Sultan food additive was assessed by thromboelastogram (TEG) recordings obtained from a thromboelastograph (Tromb-2). The following parameters were recorded in the thromboelastograms: R-time – the time from the start of the recording to the separation of the TEG edges by 1 mm. This parameter is the blood reaction time, which characterizes stages I and II of the blood coagulation process. K – the time from the end of the reaction time to the separation of the TEG edges by 20 mm. This parameter is called the time of blood clot formation or the thromboelastographic constant of thrombin and this parameter depends on the concentration of thrombin formed and the amount of fibrinogen. MA – the maximum amplitude, which is affected by the concentration of fibrinogen, the number and quality of platelets.

In chronic toxicity studies, a simple and convenient "open field" test was used to assess the effects of the study drug on the behavior of animals. In this test Glyrutin The effect of chronic administration on the number of animal movements and their qualitative composition was evaluated. The "Open field" test studies how an animal behaves when placed in an unfamiliar open field from which it cannot leave. The experiments were carried out in the Metabolomics Laboratory in a 1 m^2 area device for rats, surrounded by opaque 20 cm high barriers. This device is divided into 16 squares and contains 16 holes.

Toxicity and cumulative properties of As-Sultan food additive

As-Sultan food supplement acute toxicity According to the results of the experiment, animals administered this drug at doses of 4000, 5000 and 6000 mg/kg did not show clinical signs

of poisoning and no changes in their behavior were observed. Clinical signs of poisoning were observed in the first 5-15 minutes in animals administered doses of 8000, 10000 mg/kg. After the introduction of the sample, a decrease in the motor activity of the animals and their huddling were observed. The motor activity of the animals was restored on average 20-30 minutes after the introduction of the drug, depending on the dose, and the dose dependence was observed in the severity or mildness of the clinical signs of poisoning. No deaths were recorded in the animals even after a long period of time. The results obtained are presented in Table 1.

Even at the highest doses of 8000, 10000 mg/kg, which can be administered to animals, no deaths were observed. No animal deaths were recorded during the entire experiment, so it was not possible to accurately calculate the average lethal dose (LD_{50}) for a single oral administration. This indicator As-Sultan food supplement The maximum dose at which death was not observed was considered to be higher than the maximum dose possible, and the LD_{50} was >10,000 mg/kg. As-Sultan food supplementDue to the lack of direct literature data on the nature of acute poisoning sample was studied the acute toxicity of rutin to compare it with the results. The median lethal dose (LD_{50}) of rutin is also As-Sultan Food Supplementas high as 10,000 mg/kg.

Table 1

As-Sultan food supplement "Acute poisoning" indicators at various doses

Animal type: mouse	Gender	Doses, mg/kg	Number of animals/ death toll	LD_{10}	LD_{16}	LD_{50}	LD_{84}
Al-Sultan sample	Male/ female	4000	6/0			≥10000 mg/kg	
		5000	6/0				
		6000	6/0				
		8000	6/0				
		10000	6/0				
Control	Male/ female	water	6/0	-	-	-	-

As-Sultan Food Supplement When administered once to mice at the doses studied, no significant change was noted in their daily food and water intake compared to controls (Table 2).

Table 2.

As-Sultan food supplement Average body weight changes at different doses (mice, n=6)

Doses, mg/kg	The mice during the experiment averaged body weight, g			Weight gain, %
	Initial	7 days	14 days	
Men				
4000	14.6±0.64	28.2±0.64	31.5±1.15	33.4
6000	25.9±0.75	29.9±0.51	29.9±0.67	31.9
8000	23.4±0.37	26.1±0.29	21.8±0.37	38.1
10000	20.2±0.64	30.9±0.75	27.9±0.64	32.7
control	20.6±0.84	23.7±0.66	22.6±0.41	35.6
Females				
4000	19.7±0.42	25.4±0.25	25.9±0.27	31.7
6000	22.9±0.67	25.1±0.39	27.4±0.36	35.8
8000	21.3±0.43	27.8±0.61	30.8±0.43	37.3
10000	23.3±0.50	26.7±0.48	28.1±0.67	35.8
control	24.5±0.44	25.8±0.31	29.5±0.49	30.1

The dynamics of body mass growth in the sampled animals was positive, the rate of body mass gain did not differ from that in the control group (r>0.05).

As-Sultan food supplement the experimental scheme and results of the cumulation experiment are presented in Table 3. The total dose of glirutin administered to mice for 28 days was 11200 mg/kg, and no animal deaths were observed until the end of the experiment, and the median lethal dose for multiple administration was LD_{50_n}>11200 mg/kg. Taking into account the

above results, the cumulation coefficient was calculated based on the median lethal dose of LD_{50_1} >10000mg/kg determined for acute toxicity of the drug after a single administration.

$$K_k = LD_{50\_n} / LD_{50\_1} = 11200/10000 = 1,12$$

K_k- cumulation coefficient;

LD_{50_n} - average lethal dose after multiple administration;

LD_{50_1} - median lethal dose after a single injection;

According to the calculated result, K_k was >1, which shows that As-Sultan Food Supplementdoes not have a cumulative property.

Table 3

The results of the study of the cumulative properties of As-Sultan food additive in mice

Entry dates	Number of animals, n=10	The fraction obtained from LD_{50}	LD_{50_1}=10000 mg/kg
1-4	0/10	0.19	1000
5-8	0/10	0.21	1500
9-12	0/10	0.29	2200
13-16	0/10	0.42	3400
17-20	0/10	0.59	5000
21-24	0/10	0.75	7500
25-28	0/10	1.64	11200

CONCLUSION

Results of the experiments carried out As-Sultan food supplement showed that the median lethal dose LD_{50}>10000 mg/kg, and when classified according to acute toxicity, it was determined that the chemical compounds belong to the relatively harmless class VI. According to the calculated result, K_k>1, which shows that As-Sultan Food Supplementdoes not have a cumulative property.

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