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THE STUDY OF ACUTE, SUBACUTE TOXICITY AND CUMULATIVE PROPERTIES OF *BIDENS FRONDOSA* L., HARVESTED IN UZBEKISTAN

***Actuality**. In the modern world, herbal medicines are used for the prevention and treatment of various diseases, along with a wide range of synthetic medicines. Currently, high quality standards are applied to all stages of the creation of pharmacological products, from research and development to implementation and production. Special attention is paid to ensuring the reliability of preclinical trials and ensuring the safety of new drugs.*

Bidens tripartita L. is a popular medicinal plant included in the State Register of Medicines of the Republic of Uzbekistan. However, due to the reduction of the natural habitats of the plant, the processing enterprises of the Republic began to experience a significant need for raw materials of this species, which is not yet cultivated in Uzbekistan. Due to the decrease in the area of growth, traditionally used in medical practice of the species of Bidens tripartita L., it was advisable to conduct a comprehensive pharmacognostic study of the Bidens frondosa L. species endemic to the Republic of Uzbekistan, which has significant reserves of medicinal plant raw materials.

The aim. The objective of the research was to conducting pharmacological studies of acute and subacute toxicity and cumulative properties of Bidens frondosa L., harvested in Uzbekistan.

***Material and methods.** The experiment was carried out according to the recommendations for conducting preclinical studies of medicines. Acute toxicity was studied in white mongrel mice, with a single injection of the test sample with the determination of LD_{50} and toxicity class. Experiments to study subacute (subchronic) toxicity were carried out on 80 rats (40 males and 40 females) of the same age and body weight 170–200 g. The animals were kept in vivarium conditions with a standard diet (free access to water and feed), temperature and light conditions. All manipulations with animals were performed at the same time of day in the morning, taking into account the chronobiological dependence of most physiological processes in the body. The registration of biochemical blood parameters was carried out using standard reagent kits on a HumaLuzerPrimus 602828 biochemical analyzer, Germany.*

An experiment to study the cumulative properties of the medicinal plant Bidens frondosa L., was carried out according to the method of Lim R. et al. on 10 white mice weighing 20–22 g.

***Research results.** As a result of the study of acute toxicity, it was found that the herb of the Bidens frondosa L., does not cause violations of the functional state of the main organs and systems of the body, and also does not have a general toxic effect at the studied doses and routes of administration. The data obtained from the study of subacute toxicity showed that after stopping the administration of herbal infusion of the Bidens frondosa L., death of rats was not observed in any of the groups. The observed changes in the clinical*

manifestations and behavioral reactions of mice during the experiment were reversible, and the animals were physiologically fully restored. No animal deaths were observed during the experiment.

Conclusion. *According to the classification of toxicity of substances, aboveground part of Bidens frondosa L., refers to practically non-toxic. In the study of subacute toxicity, the medicinal plant Bidens frondosa L., does not cause violations of the functional state of the main organs and systems of the body. It does not have a general toxic effect at the studied doses and routes of administration. The medicinal plant Bidens frondosa L., does not have cumulative properties.*

Key words: *Bidens frondosa L., aboveground part (herb), infusion, acute and subacute toxicity, cumulative properties.*

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ДОСЛІДЖЕННЯ ГОСТРОЇ, ПІДГОСТРОЇ ТОКСИЧНОСТІ ТА КУМУЛЯТИВНИХ ВЛАСТИВОСТЕЙ *BIDENS FRONDOSA* L., ВИРОЩЕНОЇ В УЗБЕКИСТАНІ

Актуальність*. У сучасному світі рослинні ліки використовуються для профілактики та лікування різних захворювань, поряд із широким спектром синтетичних ліків. Натепер високі стандарти якості застосовуються на всіх етапах створення фармакологічних продуктів, від досліджень і розробок до впровадження та виробництва. Особлива увага приділяється забезпеченню надійності доклінічних випробувань та гарантуванню безпеки нових ліків.*

Bidens tripartita L. – популярна лікарська рослина, включена до Державного реєстру лікарських засобів Республіки Узбекистан. Однак через скорочення природних ареалів рослини переробні підприємства Республіки почали відчувати значну потребу в сировині цього виду, яка не культивується в Узбекистані. Через зменшення площі зростання виду Bidens tripartita L. було доцільно провести комплексне фармакогностичне дослідження виду Bidens frondosa L., ендемічного для Республіки Узбекистан, що має значну сировинну базу.

Мета дослідження *– вивчення гострої, підгострої токсичності та кумулятивних властивостей Bidens frondosa L., вирощеної в Узбекистані.*

Матеріал і методи*. Експеримент проводився відповідно до рекомендацій щодо проведення доклінічних досліджень лікарських засобів. Гостру токсичність вивчали на білих мишах шляхом одноразового введення досліджуваного зразка із визначенням LD_{50} та класу токсичності. Експерименти з вивчення підгострої (підхронічної) токсичності проводили на 80 щурах (40 самців і 40 самок) одного віку й маси тіла 170–200 г. Тварин утримували в умовах віварію зі стандартним раціоном (вільний доступ до води й корму), температурними та світловими умовами. Усі маніпуляції із тваринами проводилися в той самий час – уранці, з урахуванням хронобіологічної залежності більшості фізіологічних процесів в організмі. Реєстрація біохімічних показників крові проводилася за допомогою стандартних наборів реагентів на біохімічному аналізаторі "HumaLuzerPrimus 602828" (Німеччина). Експеримент із вивчення кумулятивних властивостей трави Bidens frondosa L. проводився методом Lim R. et al. на 10 білих мишах вагою 20–22 г.*

Результати дослідження. *У результаті дослідження гострої токсичності було встановлено, що трава Bidens frondosa L. не спричиняє порушень функціонального стану основних органів і систем організму, а також не має загального токсичного впливу за досліджуваних доз і шляхів уведення. Дані, отримані в результаті дослідження підгострої токсичності, показали, що після припинення введення настою трави Bidens frondosa L. смерть щурів не спостерігалася в жодній із груп. Спостережувані зміни у клінічних проявах і поведінкових реакціях щурів під час експерименту були оборотними, і тварини повністю відновилися фізіологічно. Під час експерименту не було зафіксовано смертей тварин.*

Висновок. *За класифікацією токсичності речовин трава Bidens frondosa L. належить до практично нетоксичних. У дослідженні підгострої токсичності Bidens frondosa L. не спричиняє порушень функціонального стану основних органів і систем організму. Вона не має загального токсичного впливу за досліджуваних доз і шляхів уведення. Лікарська рослина Bidens frondosa L. не має кумулятивних властивостей.*

Ключові слова: *Bidens frondosa L., трава, настій, гостра та підгостра токсичність, кумулятивні властивості.*

Introduction. Actuality. In the modern world, herbal medicines are used for the prevention and treatment of various diseases, along with a wide range of synthetic medicines. In recent decades, there has been an increase in their use not only in the countries of Central Asia, where they have traditionally been used for many centuries, but also in Europe, the United States and other countries of the world community. The popularity of herbal medicines is primarily explained by their relative safety compared to drugs of synthetic or biotechnological origin, however, despite the apparent harmlessness of the use of medicinal plants and their preparations, the assessment of their effectiveness and safety is quite relevant (General Guidelines for Methodologies, 2000; Directive, 2004; Fan, 2012).

Currently, high quality standards are applied to all stages of the creation of pharmacological products, from research and development to implementation and production. Special attention is paid to ensuring the reliability of preclinical trials and ensuring the safety of new drugs. Most of the side effects of medicines can be prevented by taking into account the data obtained from various experiments, including laboratory tests. In addition, animal experiments play an important role in ensuring the safety of clinical trials and subsequent medical use of new drugs (Bush, 2007; Brendler, 2009; Claeson, 2014).

Until the middle of the 80s of the last centuries, the herb of the Beggarticks (*Bidens tripartipa* L.) was the only representative of the genus *Bidens* L. in the flora of Uzbekistan. Data from recent floral studies have shown that *Bidens frondosa* L., considered an invasive species in many regions of Eastern Uzbekistan, is actively settling in the vicinity of the city of Tashkent and other adjacent habitats Europe, Russia and Central Asia. Observations have shown that the natural populations of the *Bidens tripartipa* L. have been preserved only in the remote mountainous regions of the Western Tien Shan and Pamir-Alai (Abdullaeva, 2025; Shawon, 2025).

Bidens tripartita L. is a popular medicinal plant included in the State Register of Medicines of the Republic of Uzbekistan (The State Register, 2025). However, due to the reduction of the natural habitats of the plant, the processing enterprises of the Republic began to experience a significant need for raw materials of this species, which is not yet cultivated in Uzbekistan.

Due to the decrease in the area of growth, traditionally used in medical practice of the species of *Bidens tripartita* L., it was advisable to conduct a comprehensive pharmacognostic study of the *Bidens frondosa* L. species endemic to the Republic of Uzbekistan, which has significant reserves of medicinal plant raw materials. The identification of the plant according to the herbarium sample was carried out by I. I. Maltsev – candidate of biological sciences, senior researcher at the Laboratory of "Plant Resources" of the Institute of Botany of the Academy of Sciences of the Republic of Uzbekistan.

Bidens frondosa L. – an alien species of a *Bidens* that originated in North America, and is now widespread throughout Europe and Central Asia. According to folk medicine, *Bidens frondosa* L., has anti-inflammatory properties and is widely used in the treatment of various diseases (Abdullaeva, 2025; Shawon, 2025). It became obvious that in order to conduct effective and safe phytopharmacotherapy, it is necessary to have information not only about the effectiveness of the drug, but also about the possible adverse effects of this therapy. This, in turn, dictates the need for a more in-depth study of not only the pharmacological activity, but also the safety of herbal medicines at the stage of their preclinical study. It has been shown that *Bidens frondosa* L. contains phenolic compounds, including flavonoids, polysaccharides, essential oils, as well as biologically active compounds of other classes in minor amounts. In preclinical studies, the pharmacological activity of aqueous, alcoholic, methanol extracts, their subfractions, as well as individual components of *Bidens frondosa* L. is shown (Heng, 2017; Abdullaeva, 2025; Shawon, 2025).

When studying the chemical composition of *Bidens frondosa* raw materials, the correspondence of active substances such as polysaccharides, phenolic compounds (chalcones, aurones) with the chemical composition of *Bidens tripartita* herb was established, which corresponds to Pharmacopoeia article № 42 UZ-0349

Herba Bidentis. Numerical indicators of raw materials and the content of polysaccharides in *Bidens frondosa* L., were carried out according to Pharmacopoeia article 42 Uz-0349 at the "Scientific Center for Standardization of Medicines" LLC at the Tashkent Pharmaceutical Institute.

Due to the lack of information on acute and subacute toxicity, as well as cumulative properties of the aboveground part of the *Bidens frondosa* L., flooded in Uzbekistan, this study of the object remains relevant.

The aim of the study. The objective of the research was to conducting pharmacological studies of acute and subacute toxicity and cumulative properties of Bidens frondosa L., harvested in Uzbekistan.

Materials and research methods. The object of research was collected in the budding and flowering phase (late august and early September 2023), the aboveground part (herb), annual herbaceous plants of *Bidens frondosa* L., in places of its natural growth – on the territory of the Kibrai district of the Tashkent region.

All research work with laboratory animals is carried out in accordance with generally accepted ethical standards for the treatment of animals, based on standard operating procedures adopted by the research organization, which comply with the rules adopted by the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (ETS 123) (European Convention, 1986).

The acute toxicity of the test agent was studied by the generally accepted method described in the literature, a single injection of drugs with the determination of LD_{50} and toxicity class (Dunnick, 2012; Guideline on the assessment, 2017; Brondani, 2017; Huang, 2020).

For the experiment, white mongrel mice of males and females in the amount of 36 heads, body weight 19–21 g, quarantined for 14 days were used.

For the research of acute toxicity and determination of LD_{50} an aqueous infusion was obtained by maceration with a concentration of 7,5% were prepared from the compared preparations at a rate of 3 g of raw materials per 40 ml of purified water in order to conduct the study with considering the coefficient of water absorption. Since *Bidens frondosa* herb and *Bidens tripartita* herb have a similar chemical composition of biologically active substances, the aqueous extract of *Bidens frondosa* herb can serve as an analog to the aqueous extract of *Bidens tripartita* herb.

An experiment to study the acute toxicity of the compared drugs was carried out in two series. In the first series of the experiment, white mice were intragastrically injected with an aqueous infusion of the *Bidens frondosa* L., as follows:

Group 1 (6 mice) – per os at a dose of 1 500 mg/kg (0,4 ml);

Group 2 (6 mice) – per os at a dose of 2 250 mg/kg (0,6 ml);

Group 3 (6 mice) – per os at a dose of 3 000 mg/kg (0,8 ml).

In the second series of the experiment, an aqueous infusion of "Herbae Bidentis", produced by "ZAMONA RANO" LLC (Uzbekistan) was intragastrically administered as follows:

Group 1 (6 mice) – per os at a dose of 1 500 mg/kg (0,4 ml);

Group 2 (6 mice) – per os at a dose of 2 250 mg/kg (0,6 ml);

Group 3 (6 mice) – per os at a dose of 3 000 mg/kg (0,8 ml).

Experiments to study the subacute (subchronic) toxicity of the studied medicinal plant herb of *Bidens frondosa* L., were carried out on 80 (40 males and 40 females) rats of the same age and body weight 170–200 g. The experiment was carried out according to the recommendations for conducting preclinical studies of medicines (Guideline on the assessment, 2017; Brondani, 2017; Huang, 2020). The animals were kept in a vivarium with a standard diet, temperature and light conditions, with free access to water and feed. All manipulations with animals were performed at the same time of day in the morning, taking into account the chronobiological dependence of most physiological processes in the body.

For the experiment, the rats were divided into 4 groups of 10 males and 10 females for each test dose and control dose. The choice of doses was determined by the requirements of methodological recommendations and literature data (Guideline on the assessment, 2017). The investigational medicinal raw material of *Bidens frondosa* L., in doses of 100 mg/kg (conditional therapeutic), 500 mg/kg (maximum daily) and 2 500 mg/kg (maximum), was administered daily intragastrically in the form of an aqueous infusion (1:20) using a metal probe. The calculation of doses was carried out taking into account the content of active substances. The total follow-up period was 28 days. For 28 days, 20% aqueous infusion *Bidens frondosa* L., was administered intragastrically to experimental animals daily, and then the condition of the animals was monitored.

The toxic effect of the herb was judged by its general condition – survival, appearance (daily examination – the condition of the coat and skin, mucous membranes), behavior (daily), body weight change (1 time per week), food and water consumption (weekly), hematologi-

cal and biochemical blood parameters, macroscopic detectable changes in internal organs and their mass coefficients. To analyze hematological and biochemical parameters, biological material (blood) was collected according to standard methods in the morning after 14–15 hours of fasting (Ifeoma, 2013; Guideline on the assessment, 2017). The study of peripheral blood parameters was carried out before the start of the experiment, on the 14th and 28th days. The registration of biochemical blood parameters was carried out using standard reagent kits on a biochemical analyzer "HumaLuzerPrimus 602828" (Germany). The content of leukocytes, erythrocytes, platelets, hemoglobin level, hematocrit and leukocyte formula (percentage of lymphocytes, monocytes, eosinophils and granulocytes) were determined in the blood. The following parameters were measured in blood serum: total protein, albumin, urea, creatinine, glucose, triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and calcium (Ca^{2+}). After the completion of the study (day 28), all surviving animals, including controls, were humanely euthanized and then underwent necropsy. During the necropsy, the lungs, heart, liver, kidneys, spleen, adrenal glands, and thymus were collected. The masses of these internal organs were measured using analytical scales (Ifeoma, 2013).

An experiment to study the cumulative properties of the medicinal plant herb of the *Bidens frondosa* L., was carried out according to the method of Lim R. et al. on 10 white mice weighing 20–22 g. White mice were intragastrically injected with 1% aqueous infusion of the *Bidens frondosa* L., 0,08 ml was taken for the minimum dose. Then, every next 4 days, the administered doses were increased 1,5 times from the previous daily doses. The records were kept on the basis of the general clinical and physiological condition of the mice.

Research results and their discussion. When studying the acute toxicity of the aqueous infusion of the *Bidens frondosa* L., the following data were obtained:

Group 1 (dose 1 500 mg/kg): after administration of the infusion during the day, the mice remained active, there were no visible changes in behavior and functional state. The condition of the coat and skin was normal without changes, food and water were not refused, and the death of mice was not observed. On the second day and in the subsequent period of observation, there were no pathological changes in the behavior and physiological parameters of the mice. The consumption of water and feed is normal, there was no lag in growth and development. There were no deaths of mice within 14 days.

Group 2 (dose 2 250 mg/kg): after administration of the infusion during the day, the mice were active, there were no visible changes in behavior and functional status. The condition of the coat and skin was normal without changes, food and water were not refused, and the death of mice was not observed. On the second day and in the subsequent period of observation, there were no pathological changes in the behavior and physiological parameters of the mice. The consumption of water and feed is normal, there was no lag in growth and development. There were no deaths of mice within 14 days.

Group 3 (dose 3 000 mg/kg): after administration, short-term lethargy and inactivity were observed in mice, which passed after 30–40 minutes. After 1 hour, the mice returned to their previous state, their behavior was active, and their physical indicators did not deviate from the norm (table 1).

On the second day and during the entire observation period for 14 days, no changes were observed in the behavior and other physical parameters of the mice, the mice willingly consumed food and water, reactions to light and sound stimuli remained normal, hair and skin were clean, urination and fecal excretion were normal, the weight and height of the mice did not lag behind in development. No deaths of mice were observed.

Similar data were obtained in the study of acute toxicity of the infusion of "Herba Bidentis", produced by "ZAMONA RANO" LLC, Uzbekistan.

The LD_{50} studied of the infusion of *Bidens frondosa* L., was a dose of >1 500 mg/kg. The LD_{50} of the infusion of "Herba Bidentis", produced by "ZAMONA RANO" LLC, Uzbekistan, was a dose of >1 500 mg/kg.

Table 1

Determination of acute toxicity (LD_{50}) of the studied infusion of the *Bidens frondosa* L. and "Herba Bidentis", produced by "ZAMONA RANO" LLC

№ groups	Infusion of the Bidens frondosa L.				"Herba Bidentis", produced by "ZAMONA RANO" LLC			
	doses		the way of introduction	the number of dead mice	doses		the way of introduction	the number of dead mice
	mg/kg	ml			mg/kg	ml		
1	1 500	0,4	per os	0/6	1 500	0,4	per os	0/6
2	2 250	0,6	per os	0/6	2 250	0,6	per os	0/6
3	3 000	0,8	per os	0/6	3 000	0,8	per os	0/6
LD_{50}	>3 000 mg/kg				>3 000 mg/kg			

According to the classification of toxicity of substances, the compared drugs are low-toxic (Ifeoma, 2013; Guideline on the assessment, 2017).

In the study of the subacute toxicity of the studied herb of the *Bidens frondosa* L., after discontinuation of its administration, the death of rats was not observed in any of the groups. The dynamics of changes in body weight, both males and females, was generally positive, the rate of body weight gain did not differ significantly from the control group (P > 0,05) (table 2). There were no significant changes in the amount of feed and water consumed compared to control animals.

The results of peripheral blood studies showed that on the 14th day and on the 28th day after daily intragastric administration of the introductory infusion of the herb of *Bidens frondosa* L. in doses of 100 mg/kg; 500 mg/kg and 2 500 mg/kg, there were significant changes in the erythrocyte and leukocyte formulas, as well as in the number of platelets compared to it was not observed with the control (table 3).

The study of the main biochemical parameters of blood serum on the 14th day and on the 28th day after daily intragastric administration of the introductory infusion of the *Bidens frondosa* L., in doses of 100 mg/kg; 500 mg/kg and 2 500 mg/kg did not reveal significant changes compared with the data of the control group (table 4).

When studying the effect of an aqueous infusion of the studied herb of the *Bidens frondosa* L. at doses of 100 mg/kg; 500 mg/kg and 2 500 mg/kg on diuresis and

Table 2

The change in body weight of white rats with repeated administration of the introductory infusion of the *Bidens frondosa* L., g (M±m)

Terms of the study	Control	Infusion of the *Bidens frondosa* L.		
		100 mg/kg	500 mg/kg	2 500 mg/kg
Before the experience	182,4±4,2	183,3±3,7	183,6±3,4	184±3,5
14 days	190,5±5,2	191,2±4,2	193,2±4,2	194,5±4,2
28 days	200,8±4,6	200,5±5,6	202,5±3,7	203,2±3,8

Table 3

Biochemical parameters of the blood serum of white rats with repeated administration of the *Bidens frondosa* L. (M±m)

Terms of the study	Control	100 mg/kg	500 mg/kg	2 500 mg/kg
White blood cells, 10^9/l (WBC)				
14 days	7,0±0,2	6,2±0,3	6,3±0,2	6,4±0,2
28 days	7,2±0,2	6,3±0,2	6,2±0,3	6,5±0,2
Lymphocytes, % (Lym)				
14 days	68,5±4,2	67,5±3,2	66,4±4,6	66,5±4,2
28 days	70,3±3,8	66,2±3,4	65,2±3,5	66,4±3,8
Monocytes, % (Mon)				
14 days	2,2±0,1	2,0±0,1	1,9±0,1	2,0±0,1
28 days	2,0±0,2	2,0±0,1	1,9±0,1	2,0±0,1
Eosinophils, % (Eos)				
14 days	1,55±0,2	1,51±0,2	1,5±0,1	1,5±0,1
28 days	1,52±0,2	1,5±0,1	1,48±0,1	1,47±0,2
Basophils, % (Bas)				
14 days	0	0	0	0
28 days	0	0	0	0
Erythrocytes, 10^{-12}/l (RBC)				
14 days	7,0±0,2	7,2±0,2	7,3±0,2	7,3±0,1
28 days	7,2±0,2	7,3±0,2	7,3±0,3	7,5±0,2
Hemoglobin, g/l (HGB)				
14 days	135,7±4,3	140,5±3,5	141,5±3,2	140,4±3,4
28 days	134,5±4,5	142,2±3,5	145±3,0	145,7±3,2
Platelets, 10^9/l (PLT)				
14 days	640±4,5	645,3±4,5	645±5,5	642±4,2
28 days	642±3,2	644±5,3	645,2±4,2	646,2±4,6

creatinine clearance after 14 and 28 days, no significant changes were observed relative to the control (table 5).

After the end of the experiment, the condition of the rats: rats of correct physique, satisfactory nutrition, nasal and ear secretions were not detected. The coat is shiny, there are no foci of baldness, the teeth are preserved. The visible mucous membranes are pale in color, shiny. The genitals of males are developed correctly; there is no deformity or swelling of the limbs.

The animals were euthanized and autopsied. There were no macroscopically distinguishable signs of pathology of internal organs. The thoracic and abdominal cavities did not contain effusion. The correct position of the internal organs of the thoracic and abdominal cavities. The parietal and visceral leaves of the pleura and peritoneum are thin, shiny, smooth. The submandibular lymph nodes and salivary glands are oval in shape, pale yellow or pinkish in color, with a smooth surface, a thin capsule, not soldered together and the underlying tissues. When cutting, the cut surface is uniformly colored. The thyroid gland is reddish in color, of the usual size and shape, and of a moderately dense consistency. The thymus is triangular in shape, whitish in color, moderately dense in consistency, and of normal size. The intima of the aorta is smooth, shiny, whitish in color. The diameter of the aorta has not been changed. The leaves of the pericardium are thin, transparent, smooth. The size and shape of the heart are ordinary. The right and left ventricles contain a small

Table 4

Biochemical parameters of the blood serum of white rats with repeated administration of the *Bidens frondosa* L. (M±m)

Terms of the study	Control	100 mg/kg	500 mg/kg	2 500 mg/kg
Total protein, g/l				
14 days	82,0±3,5	78,5±4,5	78,5±3,5	80,5±3,2
28 days	82,3±3,2	80,0±3,7	77,8±4,0	78,6±4,2
Urea, mmol/l				
14 days	5,5±0,3	5,3±0,3	5,2±0,4	5,0±0,2
28 days	5,4±0,3	5,2±0,3	5,1±0,3	5,1±0,2
Creatinine, mmol/l				
14 days	2,2±0,2	2,0±0,2	2,0±0,1	1,9±0,1
28 days	2,2±0,2	2,1±0,1	2,1±0,1	2,0±0,1
Glucose, mmol/l				
14 days	4,5±0,2	4,0±0,2	4,0±0,2	4,1±0,2
28 days	4,6±0,3	4,1±0,2	4,0±0,2	4,2±0,2
Total cholesterol, mmol/l				
14 days	2,3±0,2	2,2±0,2	2,0±0,1	2,0±0,1
28 days	2,3±0,2	2,2±0,2	2,1±0,1	2,0±0,1
AlAt, Units/l				
14 days	136,7±3,7	135,5±4,5	133,5±3,5	136,5±3,5
28 days	135,5±3,5	134,2±3,2	135,4±3,3	135,5±3,5
AsAt, Units/l				
14 days	400±4,5	395,2±5,5	393,2±5,2	395,5±4,5
28 days	397,7±5,5	393,3±4,4	390,8±4,8	394,6±4,6
Alkaline phosphatase, E/l				
14 days	155,7±4,5	152,2±3,2	150,2±4,2	151,5±5,2
28ys	155,5±3,3	151±4,1	149,2±4,8	152,6±4,6

Table 5

Urine test results following repeated administration of *Bidens frondosa* L. to white rats (M±m)

Terms of the study	Control	100 mg/kg	500 mg/kg	2 500 mg/kg
Diuresis for 18h, ml				
14 days	2,3±0,4	2,5±0,5	2,4±0,3	2,3±0,2
28 days	2,5±0,2	2,6±0,3	2,3±0,3	2,2±0,2
Creatinine clearance, ml/min				
14 days	0,27±0,01	0,25±0,01	0,27±0,01	0,28±0,01
28 days	0,28±0,01	0,25±0,01	0,28±0,1	0,26±0,01

amount of dark liquid blood. The valves of the heart are thin, shiny, smooth. The heart muscle on the incision is uniform cherry-brownish in color, moderately dense. The lumen of the trachea and large bronchi is not changed, the mucous membrane is shiny, smooth, pale in color. Light airy, without seals to the touch, pale pink in color. There are areas of fullness. The mucous membrane of the esophagus is shiny, smooth, pale in color. The stomach is of an ordinary size and shape, filled with food contents. The mucous membranes of the stomach did not differ from the gastric membrane of the control groups of animals and were folded, pink, shiny. The local irritant effect of the medicinal herb of the *Bidens frondosa* L., on the gastric mucosa has not been revealed.

The mucous membrane of the small intestine is pale pink, shiny, smooth. The mucous membrane of the colon is grayish in color, shiny, smooth. The shape and size of the liver do not represent any changes. The surface of the liver is smooth, of a uniform dark red color, the capsule is thin and transparent. The liver tissue on the incision is full-blooded, moderately dense. The pancreas is flat, pale pink in color, lobed, and moderately dense in consistency. The spleen is of the usual shape, dark cherry color, moderately dense consistency. The surface of the organ is smooth, the capsule is thin. On the incision, small grayish follicles are visible on the dark red background of the spleen. The size and shape of the kidneys have not been changed. The surface of the kidneys is brownish in color, smooth, the capsule is thin, transparent, easily removable. The cortical and cerebral matter are clearly distinguishable on the organ section. The adrenal glands are rounded, pale yellow in color, with a smooth surface, moderately dense. The dark colored brain matter is clearly visible on the incision. The bladder is filled with clear urine. The mucous membrane of the bladder is smooth, shiny, pale in color.

The membranes of the brain are thin and transparent. The brain substance is of normal density, the surface of the brain is smooth. Gray and white matter are clearly visible on the frontal sections of the brain. The ventricles of the brain are of normal size, there is no expansion. Ovaries (in females) are oval, dense, cluster-shaped, gray-pink in color, compact. The testicles (males) are whitish in color, of normal size. The uterus (in females) is two-divided, the surface is smooth, dense, and the color is yellowish – pink. A cavity without contents. Measurement of the mass of internal organs after completion of the experiment showed that with repeated intragastric administration of an aqueous infusion of the herb of the *Bidens frondosa* L. – at doses of 100 mg/kg; 500 mg/kg and 2 500 mg/kg, the mass of internal organs had no significant changes relative to the control. Data on the determination of mass coefficients of internal organs are presented in table 6.

The study scheme, as well as the data obtained during the study of the cumulative properties of the studied herbs of the *Bidens frondosa* L. are presented in table 7.

During the entire period of administration of an aqueous infusion of herbs of the *Bidens frondosa* L., mice showed no signs of clinical changes, such as cyanosis of the muzzle, ears, tail and limbs. In the first eight days,

Table 6

Mass coefficients of organs with repeated administration of the herb of the *Bidens frondosa* L. to white rats, mg (M±m)

Terms of the study	Control	100 mg/kg	500 mg/kg	2 500 mg/kg
Heart				
28 days	3,3±0,02	3,2±0,02	3,1±0,05	3,0±0,05
Thymus				
28 days	1,2±0,05	1,3±0,05	1,28±0,05	1,25±0,03
Liver				
28 days	30,5±1,0	31,1±0,4	29,8±0,6	31,5±0,7
The spleen				
28 days	4,3±0,2	4,7±0,2	4,8±0,2	4,5±0,3
Bud				
28 days	7,0±0,2	7,3±0,2	7,2±0,2	7,0±0,2
The brain				
28 days	7,8±0,4	8,0±0,3	7,9±0,2	8,0±0,2
Testis				
28 days	6,6±0,2	6,7±0,2	7,0±0,1	6,8±0,2
The ovary				
28 days	0,5±0,02	0,52±0,01	0,5±0,01	0,5±0,02
Stomach				
28 days	5,8±0,1	6,0±0,2	6,0±0,1	6,1±0,1

Table 7

The cumulative properties of the herbs of the *Bidens frondosa* L.

The dose of administration	Duration of observation, day					
	1–4	5–8	9–12	13–16	17–20	21–24
Daily administered dose for 4 days	0,08	0,12	0,18	0,27	0,4	0,6
Total dose, ml	0,32	0,48	0,72	1,08	1,6	2,4
Total dose, ml	0,32	0,8	1,52	2,6	4,2	6,6
Number of fallen heads	–	–	–	–	–	–

clinically observed decreased activity, clustering of all mice for 20–30 minutes. After 45–60 minutes, all the mice became active and freely consumed food and water.

Most of the mice had loose stools. The fur of the animals remained smooth and shiny, some animals showed lethargy and low activity, refusal of water and feed. However, after 12 hours, the animals returned to normal again. The same pattern was observed on the 24th day of administration of the compared drugs. The observed changes in the clinical manifestations and behavioral reactions of mice during the experiment were reversible, and the animals were physiologically fully restored. No animal deaths were observed during the experiment. In this regard, due to the low toxicity of the aqueous infusion of the herbs of the *Bidens frondosa* L., the cumulation coefficient could not be determined.

Conclusions. Thus, the results obtained show that the studied medicinal raw material of the *Bidens frondosa* L., as well as the comparison drug "Herba Bidentis", produced by "ZAMONA RANO" LLC, was low-toxic. Also, the data obtained on the study of subchronic toxicity showed that the studied of herbs of the *Bidens frondosa* L., developed at the Tashkent Pharmaceutical Institute with repeated intragastric administration for 28 days at a therapeutic dose, at a maximum daily dose and 10 times higher than the therapeutic dose, does not cause violations of the functional state of the main organs and body systems. It does not have a general toxic effect at the studied doses and routes of administration, and also does not have cumulative properties. The results of the study allow us to recommend the drug for clinical study.

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