

# Pharmacotherapeutic efficiency of the new complex urological herbal medicine in experimental urolithiasis

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## Abstract

**Purpose:** The purpose of this study is to determine pharmacotherapeutic efficacy of complex urological herbal medicine on the course of experimental urolithiasis in laboratory animals. **Methods:** Experiments were carried out on white Wistar rats. Urolithiasis was reproduced by Higgins method by intragastric administration of Vitamin D<sub>2</sub>. Pharmacotherapeutic efficiency of aqueous extract of complex urological herbal remedy was determined using well-known methods of analysis. **Results:** Course administration of complex urological herbal remedy in experimental therapeutic dose of 10 mL/kg against the background of experimental urolithiasis has pronounced pharmacotherapeutic effect. It helps to normalize the morphofunctional state of the laboratory animals' kidneys at the stages of pathological process in comparison with the data in the control group of animals. **Conclusion:** New complex urological herbal remedy has a wide spectrum of pharmacological activity, which provides its pronounced pharmacotherapeutic efficacy in experimental urolithiasis. It is promising to develop methodological recommendations on the use of the developed herbal medicine in clinical practice for the treatment and prevention of urolithiasis.

**Key words:** Medicinal herbal remedies, medicinal plants, urolithiasis

## INTRODUCTION

According to medical statistics in recent decades, there has been a steady increase in the incidence of urological pathology in population, which is associated with one of the main reasons - inadequate effectiveness of the system of preventive care and prevention of diseases.<sup>[1]</sup>

Urolithiasis is one of the leading pathologies in the structure of urological diseases.<sup>[2]</sup> Patients with urolithiasis represent from 30 to 50% of the total number in urological hospitals.<sup>[3]</sup>

The incidence of urolithiasis disease in the world is at least 3% and continues to increase progressively.<sup>[4,5]</sup>

In its turn, an absolute number of the registered patients with nephrolithiasis in Russia increased by 34.5% in the period from 2002 to 2014.<sup>[1,6]</sup> Urolithiasis leads to long-term incapacity and disability of population. It is characterized by

relapses, which are observed in approximately 50% of cases within 5 years after the first episode of the disease.<sup>[7]</sup>

Effective therapy and prevention of urolithiasis include methods of action aimed at the elimination of etiological factors of the disease and pathogenetic conditions of calculi formation.<sup>[8,9]</sup> Therefore, multicomponent herbal medicines and collections that have comprehensive effect on the etiology and pathogenesis of urinary tract diseases have a particular interest in practice. They are used as an effective component of conservative treatment and also as monotherapy for urolithiasis prevention in the presence of risk factors.<sup>[10]</sup>

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According to the reference data, at the present time, there is the necessity to expand the range of complex remedies of Russian production. This will allow to ensure an individual approach to the selection of pharmacotherapy for urinary tract diseases and to expand conservative treatment options.

Therefore, the development and introduction of new medicinal herbal products intended for the treatment and prevention of urological diseases remain to be an urgent task.

We have developed complex urological herbal medicine for treatment and prevention of urolithiasis, consisting of medicinal vegetable raw material: Leaves of *Vaccinium vitis-idaea* L. (family *Ericaceae*), herb of *Equisetum arvense* L. (family *Equisetaceae*), roots of *Arctium lappa* L. (family *Asteraceae*), fruits of *Anethum graveolens* L. (family *Apiaceae*), and herb of *Artemisia vulgaris* L. (family *Asteraceae*). Medicinal plant raw material included in multicomponent collection has been successfully used in official medicine for a long time, as diuretic, antispasmodic, antiseptic, anti-inflammatory, and cholagogue agent.<sup>[11]</sup>

## METHODS

The experimental studies were conducted on white Wistar rats of both sexes (with the weight of 180–200 g) in the autumn–winter period. Animals were kept in the standard vivarium conditions with the same care and nutrition, light and temperature regime, with free access to food and water.

Maintenance of animals was regulated by the following documents:

- Order of the Ministry of Health of the Russian Federation No. 708N dated 23.08.2010 “On the approval of the rules of laboratory practice;”
- Good laboratory practice;

Rules adopted by the “European Convention for the Protection of Vertebrate Animals used for Experiments or for other Scientific Purposes: EST No. 123” dated 18.03.1986 (Strasbourg, March 18, 1986).

The study protocol was coordinated with the ethics commission of the Institute of General and Experimental Biology of the Siberian Branch of the RAS (Protocol No. 6 dated 25.10.2016). Animals were sacrificed by the method of instantaneous decapitation under mild ether anesthesia. Herbal collection consisting of medicinal plant raw material was the object of the study:

- *Vaccinii vitis-idaeae* folia - 30%;
- *Equiseti arvensis* herba - 30%;
- *Arctii radices* - 15%;
- *Anethi graveolentis* fructus - 15%;
- *Artemisiae vulgaris* herba - 10%.

An experimental model of urolithiasis was reproduced by intragastric administration of Vitamin D2 at the dose

of 12,000 units/kg of body weight once a day for 20 days (control) by the method developed of Higgins (1965). Simultaneously with the administration of Vitamin D2, animals of the experimental groups were intragastrically administered with a decoction of the studied herbal collection in the dose of 10 mL/kg. Simultaneously with administration of Vitamin D2, decoction of the studied collection in experimental therapeutic dose of 10 mL/kg intragastrically was administered to the animals of the test groups. Administration of the tested complex urological herbal medicine was performed once a day from the beginning of the experiment and for 20 days. Rats from the second experimental group received the “Urological collection” (JSC “Krasnogorskleksredstva”) in the dose of 10 mL/kg as the reference medicine. Decoction of the studied herbal collection and the reference remedy were prepared according to the requirements of the State Pharmacopoeia of the USSR, XI edition.<sup>[12]</sup> Animals of the control group received an equivoluminal amount of distilled water according to the similar scheme.

Functional state of the kidneys in animals was assessed by estimation of: Diuresis without load according to the conventional method and with 2.5% water load;<sup>[13]</sup> concentration of potassium, sodium, and calcium cations in the urine by the method of flame photometry on the device “Flapho-4” (Germany); and concentration of phosphorus by the method developed by Hayami Tadashi.<sup>[14]</sup> Depurination renal function was assessed by creatinine and urea contents in blood serum and urine using unified methods with the reagent kit Bio-La-Test “Lahema” (the Czech Republic); protein content in the urine was assessed by reaction with sulfosalicylic acid;<sup>[15]</sup> assessment of glomerular filtration rate was carried out according to the clearance of endogenous creatinine; and pH of the urine was assessed on the universal ionomer EV-74 (Russia) using a device for small volumes. To determine the minute urine output, the urine was collected for 3 h in the exchange cells. The latter was presented by metal cylinders with perforated bottom and a funnel designed to drain the collected urine.

For microscopy of the urinary sediment, micropreparation was prepared from the fresh urine by its centrifugation at 1,500 rpm for 10 min. After that supernatant was removed, the remaining precipitate was placed on the slide plate and examined under a microscope in 100 openings under magnification ( $\times 20$ ).<sup>[16]</sup>

To determine the concentration of malondialdehyde (MDA) in serum<sup>[17]</sup> and in the kidney homogenate,<sup>[18]</sup> a technique based on the reaction of the MDA with thiobarbituric acid and subsequent spectrophotometry of the formed colored complex was used at the wavelength of 532 nm and 580 nm.

Serum catalase activity was determined by spectrophotometric method.<sup>[19]</sup>

The obtained experimental data were statistically processed by conventional methods for a small sample with the determination of the mean value (M) and the mean error (m). Evaluation of the significance of the differences in the studies was carried out using parametric Student's t-test. Differences in mean control values and experimental data were considered statistically significant at the probability of 95% ( $P \leq 0.05$ ).

## RESULTS

The effect of the decoction of the studied herbal collection on diuretic and saluretic function of kidneys of intact rats was determined during single intragastric administration of the test substances in the volume of 10 mL/kg with 2.5% water load. Collection of the urine was carried out during 3 h after administration of phytotic drugs according to the generally accepted method. In the urine obtained, we determined the concentration of  $\text{Na}^+$  and  $\text{K}^+$  cations. The data were presented in Table 1.

As can be seen from the presented data [Table 1], a single administration of the test complex urological herbal medicine had a pronounced diuretic effect increasing excretory function of the kidneys by 91% compared to the data in the animals of the control group. At the same time, diuretic effect was much higher than that of the reference remedy - "Urological collection." Besides, single administration of decoction of the developed herbal collection in white rats showed significant influence on the excretion of sodium ions and caused the 2-fold increase in their concentration in urine compared to the data in animals of the control group. Pharmacological activity of the reference remedy was inferior to that of the studied collection. The obtained results indicated high diuretic activity of the studied herbal collection.

When determining the effect of complex urological herbal medicine on the calculi formation in the urinary tract of rats, it was established that administration of sublethal doses of Vitamin D2 was accompanied by the development of an inflammatory reaction in animal kidneys. The latter was evidenced by the presence of leukocytes and epithelium in the urine and the appearance of crystals of phosphoric acid salts in the urine sediment. It was established that course administration of the decoction of the developed herbal collection to rats in the mentioned volume limited

inflammation in the kidneys. The data obtained are given in Table 2.

It was established that the degree of leukocytes and epithelium content and the presence of phosphates in the urine increased in the control animals on day 20, while course administration of the decoction of complex urological herbal medicine to the animals in the mentioned volume caused the 3-fold reduction in the number of leukocytes and epithelium compared to the animals of the control group. At the same time, we could see almost the 2-fold reduction in the amount of phosphates. The reference medicine had unidirectional less significant influence.

Table 3 shows the results of the study of the effect of complex urological herbal medicine on the parameters of the functional state of animal kidneys in case of experimental urolithiasis induced by administration of Vitamin D2.

As follows from the presented data [Table 3], intragastric administration of sublethal doses of Vitamin D2 was accompanied by violation of the functional state of the kidneys. On day 20, diuresis in rats of the control group was reduced by 34%, while in the animals, that received decoction of the studied herbal collection and the reference remedy "Urological collection" diuresis was at the same level as in the intact group. The concentration of sodium and potassium ions was at the same level as in the animals of the intact group. At the same time, glomerular filtration rate in rats of the control group was reduced by 25%, while in animals of the experimental group, it slightly differed from animals of the intact group. By this observation period, there was an increase in the concentration of hydrogen ions in the urine of animals of the experimental group, and urine pH shifted toward acidification. At the same time, the efficacy of the reference drug was inferior to that of the developed urological herbal medicine.

Table 4 presents the results of the study related to the effect of complex urological herbal medicine on the parameters of phosphorus-calcium metabolism in case of experimental urolithiasis in white rats.

According to the presented data [Table 4], administration of Vitamin D2 was accompanied by violation of phosphorus-calcium metabolism, as indicated by a considerable increase

**Table 1:** Effect of the studied herbal collection on the rate of diuresis and the content of  $\text{Na}^+$  and  $\text{K}^+$  in the urine of white rats

Groups of animals	Parameters		
	Diuresis, mL/100 g/h	$\text{K}^+$ in the urine, mM/L	$\text{Na}^+$ in the urine, mM/L
Control ( $n=8$ )	1.17±0.033	63.9±3.83	43.5±2.61
Studied herbal collection ( $n=8$ )	2.24±0.021*	76.7±1.28*	86.9±4.34*
Urological collection ( $n=8$ )	1.6±0.014*	74.2±5.11	69.5±2.17*

\*Here and below, differences are significant in the control-experience groups at  $P \leq 0.05$ ; n: Number of animals

in phosphorus concentration in both serum and urine of the animals in the control group. At the same time in the urine, this figure was by 5.5 times higher than similar data in intact animals. It was found that course administration of water decoction of the developed herbal collection to the rats helped to reduce the level of calcium in blood serum by 27.3%. It also helped to achieve 3-fold and 1.8-fold reduction in the level of phosphorus in blood and urine, respectively, as compared to the animals of the control group.

The data given in Table 5 indicate that the administration of Vitamin D2 is accompanied by the induction of the process

of free radical oxidation of lipids and the decrease in the antioxidant status of the organism. This is evidenced by an increase in the concentration of MDA in serum and in the renal homogenate, as well as a decrease in the catalase activity in the serum of the rats of the control group.

Thus, on day 20 of the study, MDA level in the serum and kidney homogenate in the control group animals was 1.3 times higher in comparison with the similar data in intact rats. Serum catalase activity in rats of the control group decreased 1.2 times as compared to the named index in animals of the intact group.

**Table 2:** Effect of the studied herbal collection on the composition of the urine sediment of white rats with experimental urolithiasis induced by the administration of Vitamin D2 (day 20)

Parameters	Groups of animals			
	Intact	Control	Studied herbal collection	Urologic collection
Leukocytes, units per field of view	3.5±0.24	20.8±1.5	6.4±0.52*	10.5±0.24*
Epithelium, units per field of view	2.3±0.12	17.5±1.34	5.5±0.26*	8.4±0.52*
Salts (phosphates)	-	++++	++	+++

+: Indicates intensity of the presence of phosphoric acid salts in the urine sediment

**Table 3:** Influence of the studied herbal collection on the indices of functional activity of the kidneys in rats with experimental urolithiasis (day 20)

Parameters	Groups of animals			
	Intact (n=8)	Control (n=8)	Studied herbal collection (n=8)	Urological collection (n=8)
Diuresis, mL/100 g/h	1.2±0.03	0.8±0.04	1.3±0.08*	1.1±0.06*
Na <sup>+</sup> in urine, mM/L	65.2±1.74	34.8±1.86	62.8±3.14*	53.8±3.11*
K <sup>+</sup> in urine, mM/L	53.7±2.15	46.1±1.09	57.7±2.16*	51.3±2.19
GFR, µL/min	96.2±4.65	72.4±3.92	89.2±4.68*	76.5±4.23
Protein in urine, g/L	0.93±0.012	2.06±0.13	1.04±0.09*	1.25±0.14*
pH of urine, conventional units	6.7±0.25	8.8±0.56	5.9±0.46*	6.3±0.49

**Table 4:** Effect of the studied herbal collection on the concentration of calcium and phosphorus in serum and urine of white rats with experimental urolithiasis induced by administration of Vitamin D2 (day 20)

Parameters	Groups of animals (n=8)			
	Intact	Control	Studied herbal collection	Urological collection
Calcium in blood serum, µmol/mL	2.2±0.01	3.3±0.18	2.4±0.09*	2.2±0.16*
Calcium in urine, µmol/mL	2.5±0.18	3.0±0.20	2.5±0.09	2.4±0.21
Phosphorus in blood serum, µmol/mL	0.8±0.06	6.2±0.43	2.1±0.15*	2.5±0.09*
Phosphorus in urine, µmol/mL	1.5±0.01	8.3±0.86	4.5±0.32*	8.7±0.67

**Table 5:** Influence of the studied herbal collection on MDA content in blood serum and renal homogenate and serum catalase activity in experimental urolithiasis induced by administration of Vitamin D2 (day 20)

Parameters	Groups of animals (n=8)			
	Intact	Control	Studied herbal collection	Urological collection
MDA in serum µmol/L	0.42±0.025	0.56±0.026	0.47±0.009*	0.51±0.027
MDA in kidneys, nmol/g tissue	3.14±0.284	4.17±0.257	3.55±0.186*	4.09±0.264
Catalase, kU/L	54.0±2.45	44.2±3.94	52.1±2.15*	48.7±3.15



At the same time, the animals that received decoction of the studied herbal collection experienced decreases in MDA in blood serum and renal homogenate in 16% and 25% of cases, respectively, as well as an increase in the catalase activity by 15%, compared to the same parameters of the control group of animals. The reference remedy “Urological collection” was inferior by these parameters to the developed urological herbal medicine.

When examining the sectional material, it was established that no visible uroliths (magnifier) were found in all groups. Figures 1-3 show images of urine sediment microscopy.

The studies have shown that intragastric administration of Vitamin D<sub>2</sub> initiates the process of crystal formation in the urinary system in animals of the control group. It has been shown that preventive course introduction of aqueous extract of complex urological herbal medicine and reference medicine reduces the process of crystal formation.

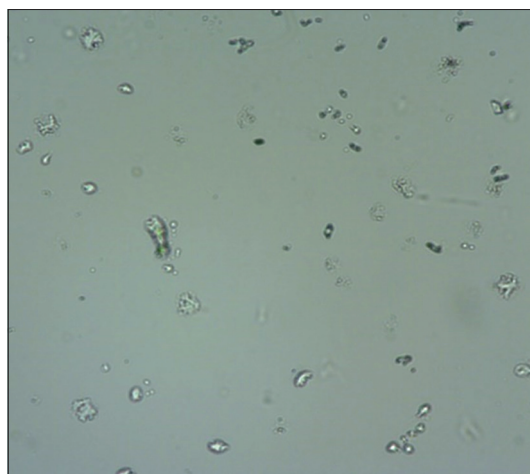
## DISCUSSION

Thus, administration of vitamin D<sub>2</sub> to the laboratory animals at the indicated dose initiates crystallization process in the urinary system accompanied by a decrease in the morphofunctional kidney condition, violation of calcium-phosphorus metabolism, an increase in the intensity of the processes of free radical oxidation and reduction in the activity of the endogenous antioxidant system of animals, which is consistent with the literature data.<sup>[13,20,21]</sup>

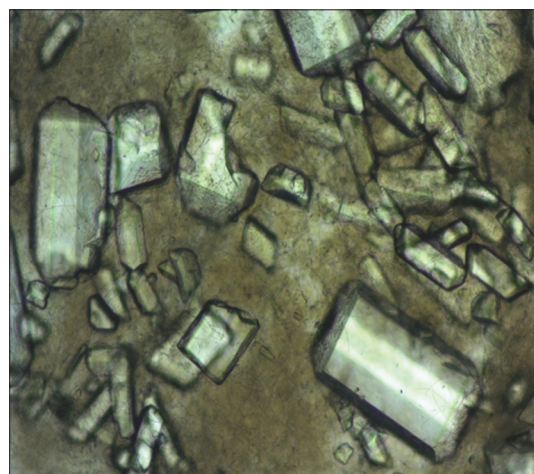
According to the evidence data, metabolic disturbances are the main pathogenetic link in the development of urolithiasis.<sup>[22,23]</sup>

We have shown that in case of experimental urolithiasis induced by the administration of sublethal doses of vitamin D<sub>2</sub>, there is a disruption of phosphorus-calcium metabolism manifested in a significant increase in the concentration of phosphorus ions in urine and serum, the appearance of phosphoric acid crystals in the urine of lab animals. Moreover, preventive-therapeutic administration of the test substance contributed to the normalization of phosphorus-calcium metabolism, as was evidenced by a decrease in the concentration of phosphorus in urine and serum, and a decrease in crystalluria.

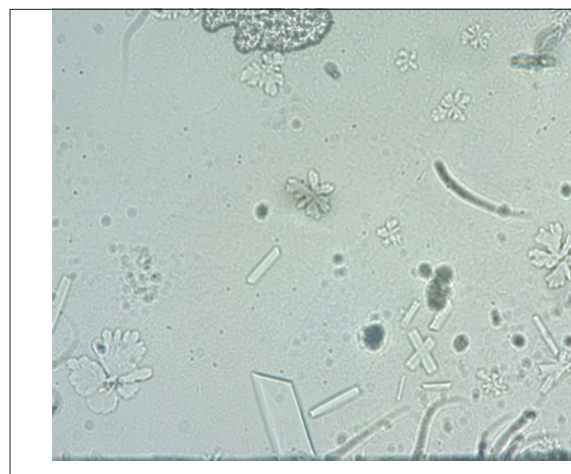
Concentration of hydrogen ions (urine pH) is a necessary condition for maintaining salts in the dissolved form. Change in the urine pH, along with an increased concentration of certain salts, can create favorable conditions for their precipitation and the subsequent formation of calculi. It was found that the alkaline reaction of urine had negative effect on the solubility of calcium phosphate salts.<sup>[2,24]</sup>



**Figure 1:** Urine sediment in rat of the intact group (×20). No uroliths found



**Figure 2:** Urine sediment in rat of the control group (×40). Crystals of tripolyphosphates (calcium phosphate)



**Figure 3:** The urine sediment of the experimental group (×20). Oxalates of calcium

One of the most important factors ensuring the realization of the pharmacotherapeutic efficacy of the complex urological

herbal remedy is the normalization of the acid-base balance, which prevents the development of alkalization of urine. The latter is one of the leading pathogenetic links in the process of calculi formation.

It can be assumed that an increase in the diuretic function of the kidneys under the influence of the test herbal collection is caused by its ability to improve intrarenal hemodynamics, as evidenced by an increase in the glomerular filtration rate.

It is known that urolithiasis is usually accompanied by the development of chronic inflammatory process in renal parenchyma, the symptoms of which are significant leukocyturia and desquamation of the tubular epithelium.<sup>[25]</sup> This is consistent with the data obtained by us. It can be assumed that anti-inflammatory activity is an important factor in the implementation of the nephroprotective effect of the complex urological herbal remedy. In particular, the use of the test substance reduced the severity of alteration and accelerated regeneration of the damaged tissues.

Free radical oxidation of lipids is one of the fundamental mechanisms of damage to biological membranes.<sup>[26]</sup> In patients with urolithiasis, urinary excretion of secondary products of lipid peroxidation (in particular, MDA), being damaging factors for renal tissue, is significantly increased. Activation of lipid peroxidation products in case of urolithiasis and the associated increase in the concentration of peroxide radicals in the intrarenal space evidently serves as one of the leading molecular-cellular mechanisms for the initiation of the process of calculi formation. As it has been established recently, free radicals, being extremely active substances, represent a nucleus for formed urinary calculi.<sup>[27]</sup>

Due to the above-noted, one can assume that the ability of the complex herbal remedy to inhibit processes of free radical lipid oxidation is one of the leading molecular-cellular mechanisms of the nephroprotective and anti-inflammatory action. It also increases the activity of the endogenous antioxidant system of the body, as evidenced by a decrease in the concentration of MDA (final peroxidation product) in the kidney homogenate and serum, as well as an increase in the activity of blood catalase.

Thus, the results of the study indicate significant pharmacotherapeutic efficacy of the new complex urological herbal remedy in case of experimental urolithiasis, as evidenced by the decrease in the intensity of the processes of calculi formation, increase in the functional consistency of the kidneys, normalization of calcium-phosphorus metabolism, and decrease in the intensity of free radical lipid oxidation processes.

Pharmacotherapeutic efficacy of the new complex urological herbal medicine was superior to the effectiveness of the reference medicine - "Urological collection" (JSC "Krasnogorskleksredstva").

## CONCLUSION

Thus, the data obtained in the work prove that the new complex urological herbal medicine has a wide range of pharmacological effects that provide its significant pharmacotherapeutic efficacy in experimental urolithiasis induced by administration of sublethal doses of Vitamin D<sub>2</sub>. The data obtained argue suitability of using the new complex urological herbal medicine in complex therapy and prevention of primary urolithiasis and recurrent calculi formation. Based on this fact, it is promising to develop methodological recommendations on the use of the developed multicomponent herbal collection in clinical practice for the treatment and prevention of urolithiasis.

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