UDC 615.322:615.451.1

Oksana KHROPOT
PhD, Department of Technology of Biologically Active Substances, Pharmacy and Biotechnology, Lviv Polytechnic National University, S. Bandery str., 12, Lviv, Ukraine, 79013 (Lvov.mp@gmail.com)
***ORCID:** 0000-0002-1985-3498*

Yulian KONECHNYI
PhD, MD, Associate Professor, Department of Microbiology, Danylo Halytsky Lviv National Medical University, Pekarska str., 69, Lviv, Ukraine, 79010 (yuliankonechnyi@gmail.com)
***ORCID:** 0000-0003-4789-1675*

Galyna LAVRYK
PhD, Senior Lecturer, Department of Microbiology, Danylo Halytsky Lviv National Medical University, Pekarska str., 69, Lviv, Ukraine, 79010 (lavrykgal@gmail.com)
***ORCID:** 0000-0002-6470-1653*

Iryna TYMCHUK
PhD, Associate Professor, Department of Microbiology, Danylo Halytsky Lviv National Medical University, Pekarska str., 69, Lviv, Ukraine, 79010 (hometira@ukr.net)
***ORCID:** 0000-0002-9290-2954*

Oleh PINYAZHKO
Doctor of Medical Sciences, Professor, Head of the Regional Department of the State Pharmacological Center of the Ministry of Health of Ukraine (Lviv region), Department of Pharmacology, Danylo Halytsky Lviv National Medical University, Pekarska str., 69, Lviv, Ukraine, 79010 (olehpinyazhko@gmail.com)
***ORCID:** 0000-0002-0961-5656*

Vira LUBENETS
Ph.D., Professor, Department of Technology of Biologically Active Substances, Pharmacy and Biotechnology, Lviv Polytechnic National University, S. Bandery str., 12, Lviv, Ukraine, 79013 (vira.i.lubenets@lpnu.ua)
***ORCID:** 0000-0001-6189-0084*

Roksolana KONECHNA
Candidate of Pharm.D., Associate Professor, Department of Technology of Biologically Active Substances, Pharmacy and Biotechnology, Lviv Polytechnic National University, S. Bandery str., 12, Lviv, Ukraine, 79013 (roksolana.t.konechna@lpnu.ua)
***ORCID:** 0000-0001-6420-9063*

To cite this article: Khropot O., Konechnyi Yu., Lavryk G., Tymchuk I., Pinyazhko O., Lubenets V., Konechna R. (2024). Doslidzhennia hostroi toksychnosti, protyzapalnoi ta hipoazotemichnoi aktyvnosti spyrtovykh ekstraktiv *Anemone nemorosa* na shchurakh [Acute toxicity, anti-inflammatory and hypoazotemic activity study of *Anemone nemorosa* acoholic extracts in rats]. *Fitoterapiia. Chasopys – Phytotherapy. Journal*, 2, 190–200, doi: https://doi.org/10.32782/2522-9680-2024-2-190

ACUTE TOXICITY, ANTI-INFLAMMATORY AND HYPOAZOTEMIC ACTIVITY STUDY OF *ANEMONE NEMOROSA* EXTRACTS IN RATS

Actuality. *The widespread use of herbal drugs with anti-inflammatory and nephroprotective properties stimulates the search for new active biological substances. Of particular interest are plants from the Ranunculaceae family, especially Anemone nemorosa, which contains a range of potentially bioactive components such as anemonin, protoanemonin, and others. Understanding the acute toxicity, anti-inflammatory, and hypoazotemic activity of Anemone nemorosa extracts opens possibilities for the development of new therapeutic agents based on this plant.*

Materials and methods. *Ethanol extracts were obtained from the aerial parts of Anemone nemorosa harvested during the flowering period. The study included the examination of oral acute toxicity conducted on Wistar rats over 14 days, anti-inflammatory activity using the carrageenan-induced paw edema method in Wistar rats, and hypoazotemic activity on models of healthy and acute renal failure in Wistar rats.*

Results. *The extracts showed no acute toxicity at the administered dose. It was established that the oral administration of the extracts is non-toxic up to a dose of 200 mg/kg body weight. The anti-inflammatory tests did not reveal significant therapeutic effects. However, the hypoazotemic tests demonstrated a reduction in blood urea and creatinine levels, and an increase in these indicators in urine, especially under conditions of acute renal failure, indicating a strong diuretic effect of the extracts.*

Conclusions. *The Anemone nemorosa extracts exhibited strong hypoazotemic and diuretic activity, which may be beneficial for the treatment of kidney diseases. The absence of anti-inflammatory activity requires further analysis and possible modification of extraction methods. The study results support the potential use of this plant in developing new nephroprotective phytopreparations.*

Key words: *Anemone nemorosa, Ranunculacea, extracts, acute toxicity, anti-inflammatory activity, hypoazotemic activity*

Оксана ХРОПОТ
доктор філософії (PhD), кафедра технології біологічно активних речовин, фармації та біотехнології, Національний університет «Львівська політехніка», вул. С. Бандери, 12, м. Львів, Україна, 79013 (Lvov.mp@gmail.com)
ORCID: *0000-0002-1985-3498*

Юліан КОНЕЧНИЙ
доктор філософії (PhD), лікар-терапевт, доцент кафедри мікробіології, Львівський національний медичний університет імені Данила Галицького, вул. Пекарська, 69, м. Львів, Україна, 79010 (yuliankonechnyi@gmail.com)
ORCID: *0000-0003-4789-1675*

Галина ЛАВРИК
кандидат біологічних наук, старший викладач кафедри мікробіології, Львівський національний медичний університет імені Данила Галицького, вул. Пекарська, 69, м. Львів, Україна, 79010 (lavrykgal@gmail.com)
ORCID: *0000-0002-6470-1653*

Ірина ТИМЧУК
кандидат медичних наук, доцент кафедри мікробіології, Львівський національний медичний університет імені Данила Галицького, вул. Пекарська, 69, м. Львів, Україна, 79010 (hometira@ukr.net)
ORCID: *0000-0002-9290-2954*

Олег ПІНЯЖКО
доктор медичних наук, професор, голова регіонального відділення Державного фармакологічного центру МОЗ України (Львівська область) кафедри фармакології, Львівський національний медичний університет імені Данила Галицького, вул. Пекарська, 69, м. Львів, Україна, 79010 (olehpinyazhko@gmail.com)
ORCID: *0000-0002-0961-5656*

Віра ЛУБЕНЕЦЬ
доктор хімічних наук, професор кафедри технології біологічно активних речовин, фармації та біотехнології, Національний університет «Львівська політехніка», вул. С. Бандери, 12, м. Львів, Україна, 79013 (vira.i.lubenets@lpnu.ua)
ORCID: *0000-0001-6189-0084*

Роксолана КОНЕЧНА
кандидат фармацевтичних наук, доцент кафедри технології біологічно активних речовин, фармації та біотехнології, Національний університет «Львівська політехніка», вул. С. Бандери, 12, м. Львів, Україна, 79013 (roksolana.t.konechna@lpnu.ua)
ORCID: *0000-0001-6420-9063*

Бібліографічний опис статті: Хропот О., Конечний Ю., Лаврик Г., Тимчук І., Піняжко О., Лубенець В., Конечна Р. (2024). Дослідження гострої токсичності, протизапальної та гіпоазотемічної активності спиртових екстрактів *Anemone nemorosa* на щурах. *Фітотерапія, Часопис*, 2, 190–200, doi: https://doi.org/10.32782/2522-9680-2024-2-190

ДОСЛІДЖЕННЯ ГОСТРОЇ ТОКСИЧНОСТІ, ПРОТИЗАПАЛЬНОЇ ТА ГІПОАЗОТЕМІЧНОЇ АКТИВНОСТІ ЕКСТРАКТІВ *ANEMONE NEMOROSA* НА ЩУРАХ

Актуальність. *Широке застосування фітопрепаратів з протизапальними та нефропротективними властивостями стимулює пошук нових активних біологічних речовин. Особливий інтерес становлять рослини родини Ranunculaceae, зокрема*

Anemone nemorosa, яка містить низку потенційно біологічно активних компонентів, як-от анемонін, протоанемонін та інші. Розуміння гострої токсичності, протизапальної та гіпоазотемічної активності екстрактів Anemone nemorosa створює можливості для розробки нових лікувальних засобів на її основі.

Матеріали та методи. *Етанольні екстракти одержували з надземної частини Anemone nemorosa, яку заготовляли в період цвітіння. Дослідження передбачало вивчення пероральної гострої токсичності, яку проводили на щурах Wistar протягом 14 днів, протизапальної активності методом карагенан-індукованого набряку задньої лапи щурів Wistar, та гіпоазотемічної активності на моделях здорових і хворих на гостру ниркову недостатність щурів Wistar.*

Результати дослідження. *Екстракти показали відсутність гострої токсичності при використанні вказаної дози. Встановлено, що при пероральному введенні екстракти є нетоксичні до рівня дози 200 мг/кг маси тіла. Протизапальні випробування не виявили значного терапевтичного ефекту. Водночас гіпоазотемічні випробування продемонстрували зниження рівнів сечовини та креатиніну в крові та збільшення цих індикаторів у сечі, особливо в умовах гострої ниркової недостатності, що свідчить про сильний сечогінний ефект екстрактів.*

Висновки. *Екстракти Anemone nemorosa виявили сильну гіпоазотемічну та сечогінну активність, що може бути корисним для лікування захворювань нирок. Відсутність протизапальної активності потребує подальшого аналізу та можливої модифікації методів екстракції. Результати досліджень підтримують потенціал використання цієї рослини для розробки нових нефропротективних фітопрепаратів.*

Ключові слова: *Anemone nemorosa, Ranunculacea, екстракти, гостра токсичність, протизапальна активність, гіпоазотемічна активність.*

Introduction. Azotemia or hyperazotemia is a biochemical condition characterized by an abnormally high level or buildup of nitrogen-containing compounds (such as urea, creatinine, various body waste compounds, and other nitrogen-rich compounds) in the blood. Azotemia is quite common; it is responsible for 8 % to 16 % of hospital admissions and, moreover, is associated with a significantly higher risk of mortality (Tyagi & Aeddula, 2019). The main cause of azotemia is a renal failure of different genesis and the following decrease of excretory renal function. Renal failure is divided into acute (ARF) and chronic (chronic kidney disease, CKD) (Akcay, Turkmen, Lee, & Edelstein, 2010). The ARF is caused by systemic diseases such as a manifestation of an autoimmune disease, e.g. lupus nephritis, crushing injury, contrast agents, some xenobiotics such as gentamicin, cysplatin and more. Very often the ARF occurs due to multiple processes. The most common cause of CKD is diabetes mellitus, followed by high blood pressure and glomerulonephritis (Vos et al., 2016). Nowadays, the prevalence of chronic and acute kidney diseases has a progressive character and is increasing, especially in developed countries. It is closely related with the spread of arterial hypertension, metabolic syndrome and diabetes mellitus etc.

According to modern conceptions, oxidative stress and inflammatory response play significant roles in the progression of ARF and CKD (Kao, Ang, Pall, & Struthers, 2010; SMALL, COOMBES, BENNETT, JOHNSON, & GOBE, 2012). The various plants and their phytopreparations with antioxidant and anti-inflammatory properties are a promising and popular source of the development of new nephroprotective drugs. Generally, the popularity of phytopreparations for the treatment of pathological processes besides the main therapeutic effect is due to a number of factors: complex effects on the body, including the normalization of vitamin balance; correction of metabolic and immunological disorders at the cellular level; low toxicity and many years of experience of ethnopharmacological uses. But the range of hypoazothemic phytopreparations is rather limited. Thus, it is necessary to search for new products on the basis of the available raw plant materials. The plants of *Ranunculaceae* family are considered to be a promising source for the development of hyperazotemic and nephroprotective phytopreparations. The *Nigella sativa* (*Ranunculaceae*) has therapeutic potential due to the presence of thymoquinone (Ahmad et al., 2013), which prevents the development of gentamicin-induced acute renal toxicity in rats (Sayed-Ahmed & Nagi, 2007). In addition, the administration of *N. sativa* extract reduces toxic effects of cisplatin in a cisplatin-induced nephrotoxicity (Hosseinian et al., n.d.); *N. sativa* oil (Bayrak et al., 2008) and ethanolic extracts (Hosseinzadeh & Montahaei, 2007) are effective free radical scavengers and protect tissues from renal ischemia/reperfusion injury. The ethanolic extract from the root of *Aconitum Heterophyllum* (*Ranunculaceae*) demonstrated good hyperazotemic activity in glycerol-induced model (Konda, 2016) and the antioxidant mechanism (increase of the superoxide dismutase, catalase and glutathione peroxidase activities) is the underlying therapeutic effect. On the other hand, plants from *Ranunculacea* are interesting as a source for phytopreparations with anti-inflammatory action due to the presence of anemonin and ranunculin, which possess anti-inflammatory properties. Due to our interest and systematic studies of pharmacological properties of *Ranunculacea* (Lukianchuk, Khropot, Konechnyi, Konechna, & Novikov, 2017), this paper is focused on the in vivo study of acute toxicity, anti-inflammatory and hypoazotemic activity of alcoholic extracts of the herb *Anemone nemorosa* (*Ranunculacea*).

Anemone nemorosa is a herbaceous perennial plant of the buttercup (*Ranunculaceae*) family. *A. nemorosa*

herb contains: alkaloids, glycosides (protoanemonin, anemonin, ranunculine, some types of saponins, tannins), vitamin C, resins, organic acids (chelidonic acid, coumarins, flavonoids, etc. Besides, γ-linolenic acid was found in seed oil. The main active substance of *A. nemorosa* is protoanemonin. During the drying of the herb, protoanemonin is converted into anemonin (Lukianchuk et al., 2017).

Materials and methods. The herb of *A. nemorosa* was collected in the Lviv Region, Scole district, Ukraine in March of 2022 and was air dried. The herb of *A. nemorosa* was used in the experiments in the form of a water-ethanol extract with code name A-2 (solvent – 40° ethanol, ratio of the raw material and ethanol – 1:40) and code name A-3 (solvent – 70° ethanol, ratio of the raw material and ethanol – 1:40). Ethanol extracts *Anemone nemorosa* L. contain phenolic compounds, flavonoids, tannins, hydroxycinnamic acids, hydroquinone derivatives, alkaloids, and anthocyanins.

Before administration to the animals, ethanol was removed from the liquid extract by evaporation in a water bath to 1/3 of the initial volume and distilled water was used to bulk the extract to the original volume.

Animals

The experiment was performed on white male and female albino Wistar rats weighing 180–220 g (for the anti-inflammatory assay, only male rats; for the acute toxicity assay, both sexes rats were present). All animals used for this study were kept in standard cages and maintained under controlled laboratory conditions of temperature (22±3°C), humidity, 12 hours day-12 hours night and had free access to food (standard pellet diet) and water ad libitum. The animals were treated humanely throughout the study period adhering to the Guide for the Care and Use of Laboratory Animals according to the Declaration of Helsinki (Bray et al., 2018; Institute of Laboratory Animal Resources (US). & Committee on Care, 1986; Suckow MA, Stevens KA, Wilson RP, 2012).

Acute toxicity

The acute toxicity study was performed on 56 albino Wistar rats of both sexes (28 males and 28 females). The animals were kept on a standard diet with free access to food and water during the experiment. The rats were allocated to 7 groups (8 animals per group). Prior to the treatment, animals were weighed, marked, and not allowed to take food overnight without suppression of water intake. Animals of the control group received distilled water, whereas in the treated groups the freshly prepared alcohol extracts from the herb of *A. nemorosa* (A-2 and A-3) were intragastrically administered with a metal probe as single doses of 5000 mg/kg, 10000 mg/kg, 15000 mg/kg body weight. The maximum dose for IV class toxicity, 5000 mg/kg, was chosen as a marker dose for acute toxicity study under the conditions of intragastric administration in accordance with the guidelines (Stefanov OV, 2001). After the administration, food was withheld for a further 3–4 h while animals were observed individually during the first 30 min, then at 2, 4, 6 h post-administration, and afterward once daily over 7 days for clinical signs of toxicity, such as mortality, respiratory pattern, changes in general behavior, skin, eyes, fur, and somatomotor activity. General characteristics of animals (eye, touch, activeness of animal, movement, etc.) before treatment and after treatment were observed without any specific scoring method or instrument involvement. The animals were observed for 14 days.

Anti-inflammatory (antiexudative) assay

40 male albino rats weighing 180–220 g were used for the antiexudative activity study. The selected animals were randomly divided into 5 groups of 8. The carrageenan-induced hind paw oedema was produced by the method originally described by Winter (Winter, Risley, & Nuss, 1962).

Carrageenan solution (1.0 % in sterile 0.9% NaCl) was injected subcutaneously into the subplanar region of the hind paw (0.1 mL in each paw) 1h after the administration of the tested extract. Diclofenac ("Diclofenac sodium", "Zdorovja narodu", Ukraine) at a dose of 8 mg/kg, Ketorolac ("Ketanov", "Terapia SA", Romania) at a dose of 10 mg/kg were used as reference drugs. Control rats received only saline solution with one drop of Tween-80™. The tested alcoholic extracts of the herb *A. nemorosa* (A-2 and A-3) were administered orally at a dose of 2 ml/kg (with one drop of Tween-80™). All reference drugs and tested extracts were injected 40 minutes before the carrageenan injection. The hind paw volume was measured with an electronic oncograph immediately before and 4h after carrageenan injection. The antiexudative activity (AEA), expressed as a decrease of the rats' paw oedema, was calculated using the equation and was expressed in percentage:

$$\text{AEA}, \% = \frac{\Delta V control - \Delta V experiment}{\Delta V control} * 100\ \%,$$

where:

$\Delta V control$ and $\Delta V experiment$ are the mean values of the volume difference for control and treated animals, respectively.

The hypoazotemic activity assay

The study of hypoazotemic activity of the alcoholic extracts from the herb of *A. nemorosa* (A-2 and A-3) was performed on healthy (intact) animals and animals with acute renal failure (ARF). The experiment involved 64 white rats that were randomly divided into groups of

8 for both stages of the experiment. The tested extracts (at a dose of 2 ml/kg) and reference drug Lespephril ("Lubnyfarm", Ukraine) at a dose of 2,0 ml/kg were orally administered for 10 days in the experiment with intact animals. After the end of the experiment the daily urine output and samples of blood from the animals were collected for biochemical tests. Acute renal failure (ARF) was modeled by a single intraperitoneal injection of mercury (II) chloride at a dose of 2 mg/kg. The tested extracts (at a dose of 2 ml/kg), reference drug Lespephril ("Lubnyfarm", Ukraine) at a dose of 2,0 ml/kg were orally administered for 10 days, starting one day before the injection of mercury (II) chloride. Animals from the "Control (ARF)" group received distilled water during the experiment. The urine samples were collected on the final day of the experiment. The animals were decapitated (under light ether anesthesia) at the end of the experiment, and samples of blood were collected for biochemical tests. The severity of ARF and therapeutic effects of the tested extracts were evaluated using urinalysis (performed using CITOLAB 11 Test (Pharmasco Ltd., Ukraine); urea and creatinine levels in serum and urine (tested using standard reagent kits from CORMAY on automatic analyzer ACCENT-200 (PZ Cormay, Poland)); sodium (Na+) levels in serum and urine (tested using Ion Selective Electrode (ISE) analysis, EASYLYTE PLUS Na/K/Cl analyzer, Medica Corp., USA).

Statistical analysis

All data were processed using the statistical software package Statistica 10.0 (Statsoft/Dell, Tulsa, OK, USA). The descriptive statistics of the data in the tables include mean ± standard error of the mean (SEM) or mean ± standard deviation. Significance was assessed by using the one-way ANOVA followed by *t*-test. Values were considered statistically significant when *P* value is less than 0,05.

Results and discussion

Acute toxicological evaluation

In the acute toxicity study, different doses of the alcoholic extracts of the herb *A. nemorosa* A-2 and A-3 were orally administered to rats for 14 consecutive days. After intragastric administration of the extracts A-2 and A-3 at a dose of 5000 mg/kg no signs of intoxication were observed in rats. The animals were clean, active, had a satisfactory appetite, responded to sound and light stimuli, urinary and defecation processes and general behavioral parameters were normal, breathing disorders and tremors were not noted (table 1).

Reflex excitability in all animals has been preserved. Immediately after the administration of the tested extracts at doses of 10000 and 15000 mg/kg short-term motor retardation of animals was observed due to the excessive fluid accumulation in the stomach, even though it was not observed at a dose of 5000 mg/kg. Subsequently, changes in appearance and behavior were not noted. Observation of the dynamics of body weight of the rats showed no differences when compared to the intact control group, and animals of both groups were evenly gaining weight (table 2). No deaths of the experimental animals were detected within 2 weeks of surveillance.

Therefore, the alcoholic extracts of herb *A. nemorosa* A-2 and A-3 at oral doses 5000 mg/kg and more show no signs of toxicity or mortality in the animals.

Anti-inflammatory activity

The carrageenan-induced hind paw oedema was used for in vivo anti-inflammatory activity evaluation of the

Table 1

Behavioral responses and general appearance of the rats treated with the alcoholic extracts of the herb *A. nemorosa* (A-2 and A-3) in acute toxicity study (M±m, n=8)

Observation	Intact animals	40 % Alcohol extract (1:40) of herb *A. nemorosa* A-2, mg/kg			70 % Alcohol Extract (1:40) of herb *A. nemorosa* A-3, mg/kg		
		5000	10000	15000	5000	10000	15000
Temperature	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Change in skin	No effect	No effect	No effect	No effect	No effect	No effect	No effect
Eye color change	No effect	No effect	No effect	No effect	No effect	No effect	No effect
Food intake	Normal	Normal	Normal	Normal	Normal	Normal	Normal
General physique	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Diarrhea	Not present	Not present	Not present	Not present	Not present	Not present	Not present
Coma	Not present	Not present	Not present	Not present	Not present	Not present	Not present
Drowsiness	Not present	Not present	Not present	Not present	Not present	Not present	Not present
Breathing difficulty	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed
Sedation	No effect	No effect	No effect	Observed	No effect	Observed	Observed
Tremor	Not present	Not present	Not present	Not present	Not present	Not present	Not present
Death	Alive	Alive	Alive	Alive	Alive	Alive	Alive

alcoholic extracts of the herb *A. nemorosa* A-2 and A-3. A strong inflammatory process with the rats' hind limb volume increases up to 125,5 % (compared to healthy conditions before the start of the experiment) was observed in the untreated group (pathology model) after 4 h after the injection of 1,0% carrageenan solution (table 3, fig. 1).

In these conditions, the reference drugs Diclofenac sodium (8,0 mg/kg) and Ketorolac (10,0 mg/kg) cause a decrease in the volume of the rat paw. The increase of the rats' hind limb volume was only 70,1 % and 79,2 % respectively, compared to healthy conditions.

Unfortunately, the tested extracts A-2 and A-3 did not reveal an anti-exudative effect in the carrageenan test in white rats. Moreover, the A-2 demonstrated slightly phlogogenic properties and provoked the development of an inflammatory process with value negative inflammation inhibition of -7,2 %. As for the extract A-3, the value of inflammation inhibition of the inflammatory reaction was very low and was only 1,83 %.

The study of hypoazotemic activity

The study of hypoazotemic activity of the alcoholic extracts of the herb *A. nemorosa* A-2 and A-3 was performed on healthy rats and on rats with acute renal failure (ARF) induced by mercury dichloride. In the experiment with healthy animals the statistically insignificant increase in 24-hour diuresis at 16,6 %, was observed in the group treated with Lespephril at a dose of 2,0 ml/kg compared to the intact group (table 4, fig. 2).

In the same conditions, the treatment by the extracts of the herb *A. nemorosa* A-2 and A 3 provoked a strong diuretic action. The total 24-hour diuresis increased up to 183 % for A-2 and 70,4 % for A-3 ($p \leq 0,05$) compared to the intact animals. A statistically insignificant decrease in urea and creatinine levels in blood serum was observed in healthy animals treated with Lespephril and the extracts A-2 and A-3 compared to the intact control group.

The decrease of the urea level in the blood was 14 % in the group treated with Lespephril, 11 % in the group treated with A-2 and 9 % in the group treated with A-3 compared to the intact animals. The creatinine level in blood was decreased slightly less than the urea level compared to the intact animals and was lowered by 11 % in the group treated with Lespephril, by 9 % in the group treated with A-2 and by only 4 % in the group treated with A-3. At this time, the urea concentration in urine was higher by 10 % in the group treated with Lespephril and by 7 % in the groups treated with A-2 and A-3 compared to intact rats. The change in creatinine concentration in urine was more distinct than the respective urea level and its concentration was higher by 34 % in the group treated with Lespephril, by 22 % in the group treated with A-2 and by 28 % in the group treated with A-3 compared to intact control group. The concentrations of ionized sodium (Na+) in the blood and urine of the experimental animals were practically unchanged in the

Table 2

Body weight assessment of the rats treated with the alcoholic extracts of the herb A. nemorosa (A-2 and A-3) in acute toxicity study (M±m, n=8)

Groups/Parameters	Doses, mg/kg	N/N'	Body weight, g			
			1 day	3 day	7 day	14 day
Intact animals	-	0/6	193±4	196±2	200±3	211±3
40 % Alcoholic Extract (1:40) of the herb *A. nemorosa* **A-2**	5000	0/6	192±3	198±3	204±3	215±3
	10000	0/6	192±3	197±3	202±3	214±3
	15000	0/6	190±2	196±2	203±2	212±3
70 % Alcoholic Extract (1:40) of the herb *A. nemorosa* **A-3**	5000	0/6	192±3	198±3	204±3	213±3
	10000	0/6	193±3	198±3	203±2	214±3
	15000	0/6	190±3	196±2	201±3	213±3

Notes: N/N'– Number of dead animals/number of surviving animals.

Table 3

***In vivo* anti-inflammatory activity of the alcoholic extracts of the herb *A. nemorosa* A-2 and A-3 on carrageenan-induced paw oedema in rats (M±m, n=8)**

Groups/Parameters	Doses	Rat hind limb volume increase, 4 hours, %	Inflammation inhibition, % (AEA)
Carragenan (Pathology model)	1 %, 0,1 ml	125,5	-
Diclofenac sodium	8,0 mg/kg	70,1	44,1
Ketorolac	10,0 mg/kg	79,2	36,9
40 % Alcoholic extract (1:40) of the herb *A. nemorosa* **A-2**	2,0 ml/kg	134,6	-7,2
70 % Alcoholic Extract (1:40) of the herb *A. nemorosa* **A-3**	2,0 ml/kg	121,8	2,9

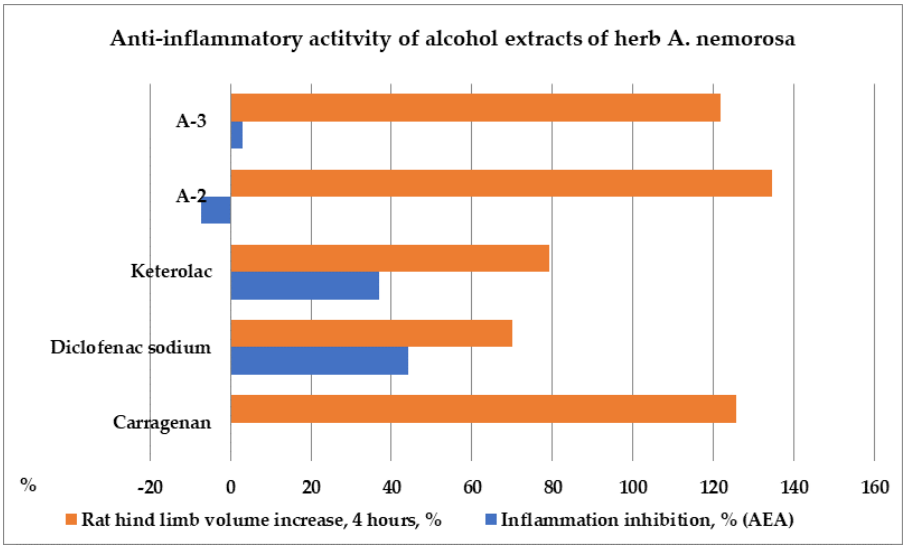

Fig. 1. *In vivo* anti-inflammatory activity of alcohol extracts of herb *A. nemorosa* A-2 and A-3 on carrageenan-induced paw oedema in rats

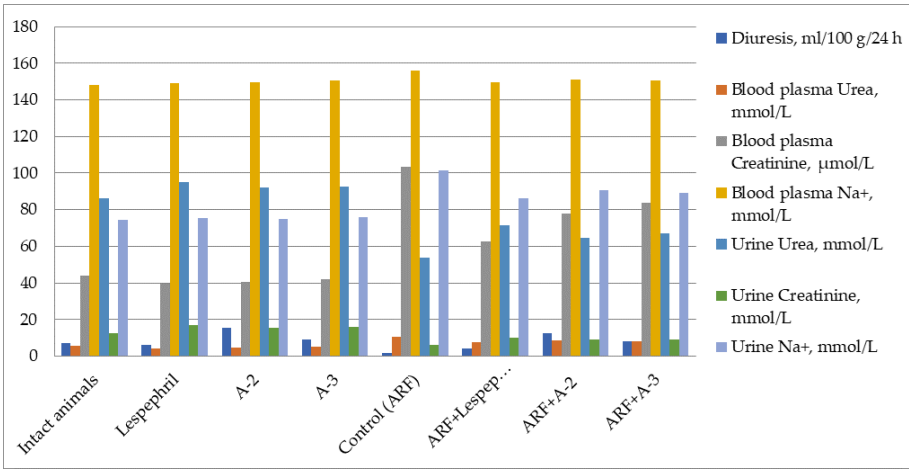

Fig. 2. Effects of the alcoholic extracts of the herb *A. nemorosa* (A-2 and A-3) on diuresis; urea, creatinine and Na+ levels in serum and urine in healthy animals and in rats with acute renal failure (ARF)

Table 4

Effects of the alcoholic extracts of the herb *A. nemorosa* (A-2 and A-3) on diuresis; urea, creatinine and Na+ levels in serum and urine in healthy animals and rats with acute renal failure (M±m, n=8)

Groups/ Parameters	Diuresis, ml /100 g / 24 h	Serum			Urine		
		Urea, mmol/L	Creatinine, µmol/L	Na^+, mmol/L	Urea, mmol/L	Creatinine, mmol/L	Na^+, mmol/L
Intact animals	5,4±0,7	5,5±0,9	43,8±3,9	147,9±3,5	86,3±5,9	12,6±2,3	74,6±3,1
Lespephril, 2,0 ml/kg	6,3±0,5	4,2±0,6$^{\#b}$	39,4±3,2$^{\#b}$	149,1±3,9	95,3±7,3$^{\#b}$	16,9±2,3*^{b}	75,3±3,0*^{b}
A-2, 2,0 ml/kg	15,3±1,4*$^{a\#b}$	4,9±0,7*^{b}	40,4±3,6$^{\#b}$	149,5±3,3	92,3±8,1*^{b}	15,4±2,3*^{b}	74,9±4,1*^{b}
A-3, 2,0 ml/kg	9,2±0,9*$^{a\#b}$	5,1±0,6*^{b}	42,2±3,1$^{\#b}$	150,4±4,1	92,7±7,9*^{b}	16,2±2,3*^{b}	75,8±4,9*^{b}
Control (ARF)	1,9±0,4*^{a}	10,7±1,3*^{a}	103,5±7,2$^{\#a}$	156,1±4,8	53,7±6,4*^{a}	5,9±1,5*^{a}	101,5±4,9$^{\#a}$
ARF+Lespephril, 2,0 ml/kg	4,0±0,6	7,8±1,0	62,7±4,7*$^{a\#b}$	149,8±4,1	71,6±6,9	10,3±1,9	86,2±4,2*^{a}
ARF+**A-2**, 2,0 ml/kg	12,5±1,6*$^{a\#b}$	8,7±0,9*^{a}	77,7±7,0$^{\#a}$	151,3±5,5	64,7±4,9*^{a}	9,2±1,7*^{a}	90,7±5,2*^{a}
ARF+**A-3**, 2,0 ml/kg	7,9±1,3$^{\#b}$	8,3±0,6*^{a}	83,9±9,4$^{\#a}$	150,7±4,8	67,3±5,1*^{a}	9,3±1,3*^{a}	89,2±4,9*^{a}

Notes: ARF – acute renal failure; * p≤0,05; #p≤0,001; a – significant compared to intact rats; b – significant compared to control rats (ARF).

Table 5

Urinalysis of rats after the administration of the alcoholic extracts of the herb *A. nemorosa* (A-2 and A-3) under the conditions of acute renal failure (M±m, n=8)

Groups / Parameters	Intact animals	Control (ARF)	Lespephril, 2,0 ml/kg	A-2, 2,0 ml/kg	A-3, 2,0 ml/kg
Specific gravity	1015±5	>1030	1020±5	1000±5	1010±5
pH	6,9±0,3	8,1±0,2	7,3±0,3	7,4±0,3	7,4±0,3
Proteine	N	N	N	N	N
Urobilinogen	N	N	N	N	N
Glucose	N	N	N	N	N
Bilirubin	N	N	N	N	N
Ketone	N	N	N	N	N
Nitrite	N	N	N	N	N
Occult blood	N	N	N	N	N

Notes: ARF – acute renal failure; N – negative.

experiment in healthy animals. Thus, the administration of the alcoholic extracts of the herb *A. nemorosa* A-2 and A-3 to healthy rats displays moderate hypoazotemic action and has a positive impact on the decrease in urea and creatinine levels in serum and the increase in the levels of these metabolites in urine. The hypoazotemic effects of the tested extracts A-2 and A-3 are practically equivalent to the effect of the plant drug Lespephril.

The administration of mercury chloride (II) solution to the laboratory animals causes the development of acute renal failure (ARF) accompanied with azotemia, oliguria, decrease of diuresis and glomerular filtration rate, an increase in the kidneys weight index with simultaneous temporal activation of lipid peroxidation processes in the kidneys. In our experiment we observed the classic symptoms and pattern of the ARF in rats on the 10th day after the injections of mercury chloride (II) solution at a dose of 2 mg/kg. The decrease of 24-hours diuresis at 65 % ($p \leq 0,05$) and a significant increase of the urine specific gravity of more than 1030 and of pH to 8,1±0,2 was observed in animals from the ARF group compared to the intact animals (Table 5). The urea and creatinine levels in serum have been significantly increased, at 94 % ($p \leq 0,05$) and at 136 % ($p \leq 0,05$) respectively, in comparison to the intact group (table 5).

At the same time, the creatinine and urea concentration in urine in the ARF group tended to decrease and was at 54 % ($p \leq 0,05$) and 38 % ($p \leq 0,05$) lower than in intact animals, which is the evidence of the kidneys' excretory function disorders of the experimental animals (Gozhenko AI, Fedoruk OS, 2002; Kemertelidze, Syrov, Alaniya, Kavtaradze, & Khushbaktova, 2008; Mariya Leleka, Olha Zalis'ka, & Galyna Kozyr, 2016; Semenyshyn, Atamanyuk, Rymar, Ivashchuk, & Hlukhaniuk, 2020). The clear disruption of the sodium ions (Na+) transport caused by mercury chloride (II) administration was manifested in the significant increase in its concentration in urine (at 36 %, $p \leq 0,001$), and a decrease in its concentration in blood.

The use of Lespephril and the extracts A-2 and A-3 had a positive effect on animals with ARF (Tables 4,5). The 24-hour diuresis in group ARF+Lespephril increased by 110 % ($p \leq 0,05$) compared to ARF group and was 26 % less than in intact animals. The administration of extracts A-2 and A-3 under the ARF conditions provoked a strong diuretic effect. The 24-hour diuresis in ARF+A-2 group was the highest at 531 % ($p \leq 0,001$) and 131 ($p \leq 0,05$) compared to the ARF group and the intact animals, respectively. In the ARF+A-3 group the 24-hour diuresis was the highest at 315 % ($p \leq 0,05$) and 26 % compared to the ARF group and the intact animals, respectively. The decrease of urine specific gravity to 1020±5 (ARF+Lespephril), 1000±5 (ARF+A-2) and 1010±5 (ARF+A-3) was observed for all animal groups which received the treatment. The normalization of the pH level was noted as well and its values were 7,3±0,3 in the ARF+Lespephril group and 7,4±0,3 in groups ARF+A-2 and ARF+A-3.

Levels of creatinine, urea and sodium ions (Na+) concentration in the serum in groups ARF+Lespephril, ARF+A-2, ARF+A-3 were lower in comparison to the level of the ARF group and higher than in intact animals. The use of Lespephril under the ARF conditions causes a decrease in the creatinine level in blood by 40 % ($p \leq 0,05$) compared to the ARF group and this level is 43 % ($p \leq 0,001$) higher than in intact animals. The urea level in the blood of an animal from the ARF+Lespephril group was lower by 27 % compared to ARF group and 41 % higher than in intact animals. The treatment by the extract A-2 decreased blood urea by 19 % compared to the ARF group and it was 11 % and 58 % ($p \leq 0,05$) higher than in the ARF+Lespephril group and the intact animals,

respectively. The creatinine blood level in the ARF+A-2 group was 25 % lower than in the ARF group and 23 % and 77 % higher than in the ARF+Lespephril group and the intact animals, respectively. The administration of the extract A-3 decreased blood urea by 23 % compared to the ARF group and it was 10 % and 50 % ($p \leq 0,05$) higher than in the ARF+Lespephril group and the intact animals, respectively. The creatinine blood level in the ARF+A-3 group was 19 % lower than in the ARF group and 33 % ($p \leq 0,05$) and 83 % ($p \leq 0,001$) higher than in the ARF+Lespephril group and the intact animals respectively. The level of sodium ions (Na+) in the blood of the treated animals had a tendency to normalize and was lower by 5 % (ARF+Lespephril) and 4 % (groups ARF+A-2 and ARF+A-3) compared to the ARF group, but higher by 1,2 %, 2,2 % and 1,8 % respectively compared to the intact animals.

The urea and creatinine levels in urine increased in groups ARF+Lespephril, ARF+A-2, ARF+A-3 in comparison to the ARF group and their values were higher than in the intact animals. The urea level in urine was 33 %, 20 %, and 25 % higher than the ARF group and 18 %, 25 % ($p \leq 0,05$), and 23 % ($p \leq 0,05$) lower compared to the intact animals in groups ARF+Lespephril, ARF+A-2, ARF+A-3 respectively. At the same time, the creatinine level in urine increased by 74%, 56 %, and 58 % compared to the ARF group and was 19 %, 27 % ($p \leq 0,05$), and 26 % ($p \leq 0,05$) lower than in the intact animals in groups ARF+Lespephril, ARF+A-2, ARF+A-3 respectively. The sodium ions (Na+) concentration in urine in the treated animals was 15 %, 11 %, and 12 % lower than in the ARF group and 16 %, 20 % ($p \leq 0,05$), and 19 % ($p \leq 0,05$) higher than in the intact animals in groups ARF+Lespephril, ARF+A-2, ARF+A 3 respectively.

The above data indicates that the hypoazotemic activity of the alcoholic extracts of the herb *A. nemorosa* A-2 and A-3 is equivalent to the Lespephril effect. The hypoazotemic activity of extracts A-2 and A-3 was more marked in the hyperazotemic process in the ARF model. It should be noted that the hypoazotemic activity of the extracts A-2 and A-3 both in the experiment in healthy animals and in the conditions of the ARF model is combined with strong diuretic action. These properties are very useful for the therapy of disorders and diseases associated with intoxication by the excess of nitrogen and when its removal from the body is necessary, such as pyelonephritis, glomerulonephritis, diabetes mellitus etc. Due to a moderate level of hypoazotemic action, the alcoholic extracts of the herb *A. nemorosa* might be used for long-term administration.

Conclusions

In conclusion, acute toxicity, anti-inflammatory and hypoazotemic activity of alcoholic extracts of the herb *A. nemorosa* was studied in vivo in white rats. It was established that the tested alcoholic extracts are non-toxic up to a dose level 15 000 mg/kg body weight. No significant anti-inflammatory effect of the alcoholic extracts of the herb *A. nemorosa* was observed in the carrageenan test. Strong diuretic and moderate hypoazotemic activities were observed after the administration of the alcoholic extracts of the herb *A. nemorosa* to healthy rats and also to the rats with acute renal failure model (ARF). The hypoazotemic action of the extracts was more marked in the hyperazotemic process in the ARF model and was equivalent to the effect of the reference drug Lespephril. The present results provide a good background for developing an individual or combined hypoazotemic plant drugs on the basis of the herb *A. nemorosa* extracts, which contain the whole complex of materials from the starting plant material and are in fact a natural product.

BIBLIOGRAPHY

Ahmad A., Husain A., Mujeeb M., Khan S. A., Najmi A. K., Siddique N. A., … Anwar F. *A review on therapeutic potential of Nigella sativa: A miracle herb.* Asian Pacific Journal of Tropical Biomedicine, 2013. 3(5), 337–352. https://doi.org/10.1016/S2221-1691(13)60075-1

Akcay A., Turkmen K., Lee D., Edelstein C. L. *Update on the diagnosis and management of acute kidney injury.* International Journal of Nephrology and Renovascular Disease, 2010. 3(2), 129–140. https://doi.org/10.2147/IJNRD.S8641

Bayrak O., Bavbek N., Karatas O. F., Bayrak R., Catal F., Cimentepe E., … Akcay A. *Nigella sativa protects against ischaemia/reperfusion injury in rat kidneys.* Nephrology Dialysis Transplantation, 2008. 23(7), 2206–2212. https://doi.org/10.1093/ndt/gfm953

Bray F., Ferlay J., Soerjomataram I., Siegel R. L., Torre L. A., Jemal A. *Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.* CA: A Cancer Journal for Clinicians, 2018. 68(6), 394–424. https://doi.org/10.3322/caac.21492

Гоженко АІ, Федорук ОС, Погоріла ІВ. *Вплив аргініну на функціональний стан нирок щурів при сулемовій нефропатії.* Фізіологічний журнал. 2002;48(6):26-30.

Hosseinian S., Khajavi Rad A., Hadjzadeh M.-A.-R., Mohamadian Roshan N., Havakhah S., Shafiee S. (n.d.). *The protective effect of Nigella sativa against cisplatin-induced nephrotoxicity in rats.* Avicenna Journal of Phytomedicine, 6(1), 44–54. URL:http://www.ncbi.nlm.nih.gov/pubmed/27247921

Hosseinzadeh H., Montahaei R. *Protective effect of Nigella sativa L. extracts and thymoquinone, its active constituent, on renal ischemia-reperfusion-induced oxidative damage in rats.* Pharmacologyonline, 2007. 1, 176–189.

Institute of Laboratory Animal Resources (US)., & Committee on Care, U. of L. A. Guide for the care and use of laboratory animals. US Department of Health and Human Services, Public Health Service, National Institutes of Health. 1986.

Kao M. P. C., Ang D. S. C., Pall A., Struthers A. D. *Oxidative stress in renal dysfunction: mechanisms, clinical sequelae and therapeutic options.* Journal of Human Hypertension, 2010. 24(1), 1–8. https://doi.org/10.1038/jhh.2009.70

Kemertelidze É. P., Syrov V. N., Alaniya M. D., Kavtaradze N. S., Khushbaktova Z. A. *Chemical composition and pharmacological activity of the leaves of Pueraria hirsuta L. grown in Georgia.* Pharmaceutical Chemistry Journal, 2008. 42(6), 340–343. https://doi.org/10.1007/s11094-008-0131-9

Konda V. G. R. *Antioxidant and Nephroprotective Activities of Aconitum heterophyllum Root in Glycerol Induced Acute Renal Failure in Rats.* Journal of Clinical And Diagnostic Research. 2016. https://doi.org/10.7860/JCDR/2016/10798.7388

Lukianchuk A., Khropot O., Konechnyi Y., Konechna R., Novikov V. *Анемона дібровна. Anetone Nemorosa L. Аналітичний огляд.* ScienceRise: Pharmaceutical Science, 2017. 3 (7), 34–38. https://doi.org/10.15587/2519-4852.2017.104438

Mariya Leleka, Olha Zalis'ka, Galyna Kozyr. *Screening Research of Pharmaceutical Compositions Based on Succinic Acid, Ascorbic Acid and Rutin.* Journal of Pharmacy and Pharmacology, 2016. 4(9). https://doi.org/10.17265/2328-2150/2016.09.003

Sayed-Ahmed M. M., Nagi M. N. *Thymoquinone supplementation prevents the development of gentamicin-induced acute renal toxicity in rats.* Clinical and Experimental Pharmacology and Physiology, 2007. 34(5–6), 399–405. https://doi.org/10.1111/j.1440-1681.2007.04560.x

Semenyshyn Y., Atamanyuk V., Rymar T., Ivashchuk O., Hlukhaniuk A. *Mass Transfer in the Solid-Liquid System: Mechanism and Kinetics of the Extraction Process.* Chemistry & Chemical Technology, 2020. 14(1), 121–128. https://doi.org/10.23939/chcht14.01.121

Small D. M., Coombes J. S., Bennett N., Johnson D. W., Gobe G. C. *Oxidative stress, anti-oxidant therapies and chronic kidney disease.* Nephrology, 2012. 17(4), 311–321. https://doi.org/10.1111/j.1440-1797.2012.01572.x

Стефанов О. В. *Доклінічні дослідження лікарських засобів: методичні рекомендації.* 2001.

Suckow M. A., Stevens K. A., Wilson R. P. (Eds.). *The laboratory rabbit, guinea pig, hamster, and other rodents.* Academic Press. 2012.

Tyagi A., Aeddula N. R. *Azotemia.* In StatPearls. 2019. URL: http://www.ncbi.nlm.nih.gov/pubmed/30844172

Vos T., Allen C., Arora M., Barber R. M., Bhutta Z. A., Brown A., … Murray C. J. L. *Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015.* The Lancet, 2016. 388(10053), 1545–1602. https://doi.org/10.1016/S0140-6736(16)31678-6

Winter C. A., Risley E. A., Nuss G. W. *Carrageenin-Induced Edema in Hind Paw of the Rat as an Assay for Antiinflammatory Drugs.* Experimental Biology and Medicine, 1962. 111(3), 544–547. https://doi.org/10.3181/00379727-111-27849

REFERENCES

Ahmad, A., Husain, A., Mujeeb, M., Khan, S. A., Najmi, A. K., Siddique, N. A., … Anwar, F. (2013). *A review on therapeutic potential of Nigella sativa: A miracle herb.* Asian Pacific Journal of Tropical Biomedicine, 3(5), 337–352. https://doi.org/10.1016/S2221-1691(13)60075-1

Akcay, A., Turkmen, K., Lee, D., & Edelstein, C. L. (2010). *Update on the diagnosis and management of acute kidney injury.* International Journal of Nephrology and Renovascular Disease, 3(2), 129–140. https://doi.org/10.2147/IJNRD.S8641

Bayrak, O., Bavbek, N., Karatas, O. F., Bayrak, R., Catal, F., Cimentepe, E., … Akcay, A. (2008). *Nigella sativa protects against ischaemia/reperfusion injury in rat kidneys.* Nephrology Dialysis Transplantation, 23(7), 2206–2212. https://doi.org/10.1093/ndt/gfm953

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). *Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.* CA: A Cancer Journal for Clinicians, 68(6), 394–424. https://doi.org/10.3322/caac.21492

Gozhenko AI, Fedoruk OS, P. I. (2002). *Vplyv arhininu na funktsional'nyy stan nyrok shchuriv pry sulemoviy nefropatiyi. [Influence of arginine on the functional state of rats kidney in Mercury(II) chloride nephropathy.]* Fisioloh Zh, 48(6), 26–30. [in Ukrainian].

Hosseinian, S., Khajavi Rad, A., Hadjzadeh, M.-A.-R., Mohamadian Roshan, N., Havakhah, S., & Shafiee, S. (n.d.). *The protective effect of Nigella sativa against cisplatin-induced nephrotoxicity in rats.* Avicenna Journal of Phytomedicine, 6(1), 44–54. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/27247921

Hosseinzadeh, H., & Montahaei, R. (2007). *Protective effect of Nigella sativa L. extracts and thymoquinone, its active constituent, on renal ischemia-reperfusion-induced oxidative damage in rats.* Pharmacologyonline, 1, 176–189.

Institute of Laboratory Animal Resources (US)., & Committee on Care, U. of L. A. (1986). Guide for the care and use of laboratory animals. US Department of Health and Human Services, Public Health Service, National Institutes of Health.

Kao, M. P. C., Ang, D. S. C., Pall, A., & Struthers, A. D. (2010). *Oxidative stress in renal dysfunction: mechanisms, clinical sequelae and therapeutic options.* Journal of Human Hypertension, 24(1), 1–8. https://doi.org/10.1038/jhh.2009.70

Kemertelidze, É. P., Syrov, V. N., Alaniya, M. D., Kavtaradze, N. S., & Khushbaktova, Z. A. (2008). *Chemical composition and pharmacological activity of the leaves of Pueraria hirsuta L. grown in Georgia.* Pharmaceutical Chemistry Journal, 42(6), 340–343. https://doi.org/10.1007/s11094-008-0131-9

Konda, V. G. R. (2016). *Antioxidant and Nephroprotective Activities of Aconitum heterophyllum Root in Glycerol Induced Acute Renal Failure in Rats.* Journal of Clinical And Diagnostic Research. https://doi.org/10.7860/JCDR/2016/10798.7388

Lukianchuk, A., Khropot, O., Konechnyi, Y., Konechna, R., & Novikov, V. (2017). *Anemona dibrovna. Anemone Nemorosa L. Analitychnyi ohliad [Wood anemone. Anemone Nemorosa L. Analytical review.]* ScienceRise: Pharmaceutical Science, (3 (7)), 34–38. https://doi.org/10.15587/2519-4852.2017.104438 [in Ukrainian].

Mariya Leleka, Olha Zalis'ka, & Galyna Kozyr. (2016). *Screening Research of Pharmaceutical Compositions Based on Succinic Acid, Ascorbic Acid and Rutin.* Journal of Pharmacy and Pharmacology, 4(9). https://doi.org/10.17265/2328-2150/2016.09.003

Sayed-Ahmed, M. M., & Nagi, M. N. (2007). *Thymoquinone supplementation prevents the development of gentamicin-induced acute renal toxicity in rats.* Clinical and Experimental Pharmacology and Physiology, 34(5–6), 399–405. https://doi.org/10.1111/j.1440-1681.2007.04560.x

Semenyshyn, Y., Atamanyuk, V., Rymar, T., Ivashchuk, O., & Hlukhaniuk, A. (2020). *Mass Transfer in the Solid-Liquid System: Mechanism and Kinetics of the Extraction Process.* Chemistry & Chemical Technology, 14(1), 121–128. https://doi.org/10.23939/chcht14.01.121

Small, D. M., Coombes, J. S., Bennett, N., Johnson, D. W., & Gobe, G. C. (2012). *Oxidative stress, anti-oxidant therapies and chronic kidney disease.* Nephrology, 17(4), 311–321. https://doi.org/10.1111/j.1440-1797.2012.01572.x

Stefanov OV. (2001). *Doklinichni doslidzhennya likarskyh zasobiv: Metodychni rekomendatsii. [Preclinical studies of drugs. Guidelines].* Kyiv. [in Ukrainian].

Suckow, M. A., Stevens, K. A., & Wilson, R. P. (Eds.). (2012). *The laboratory rabbit, guinea pig, hamster, and other rodents.* Academic Press.

Tyagi, A., & Aeddula, N. R. (2019). *Azotemia*. In StatPearls. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/30844172

Vos, T., Allen, C., Arora, M., Barber, R. M., Bhutta, Z. A., Brown, A., … Murray, C. J. L. (2016). *Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015.* The Lancet, 388(10053), 1545–1602. https://doi.org/10.1016/S0140-6736(16)31678-6

Winter, C. A., Risley, E. A., & Nuss, G. W. (1962). *Carrageenin-Induced Edema in Hind Paw of the Rat as an Assay for Antiinflammatory Drugs.* Experimental Biology and Medicine, 111(3), 544–547. https://doi.org/10.3181/00379727-111-27849

Стаття надійшла до редакції 06.03.2024.
Стаття прийнята до друку 14.05.2024.

Author Contributions: Conceptualization, R.T.; methodology, O.K.; software, G.L., I.T.; formal analysis, Y.K., G.L., I.T.; investigation, O.K., O.P.; resources, V.L.; data curation, R.T.; writing – original draft preparation, O.K.; writing – review and editing, Y.K. and R.K.; visualization, O.P.; supervision, R.K.; funding acquisition, Y.K. All authors have read and agreed to the published version of the manuscript.

Funding: The research leading to these results has received funding from the Ministry of Health of Ukraine, under project number 0123U100153.

Institutional Review Board Statement: The experiment design and study protocol were approved by the Animal Ethics Committee of the Danylo Halytsky Lviv National Medical University, protocol No. 14 from June 06, 2018.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We thank the Armed Forces of Ukraine for being able to develop science, even during the war.

Conflicts of Interest: The authors declare no conflicts of interest.

Усі автори прочитали та погодилися з опублікованою версією рукопису.

Внесок авторів:

Конечна Р. – ідея;
Хропот О. – проведення експерименту, методологія;
Лаврик Г., **Тимчук І.** – аналіз даних;
Конечна Р., Хропот О. – написання рукопису;
Конечний Ю. – фінансування, редагування тексту;
Піняжко О. – візуалізація;
Лубенець В. – ресурсне забезпечення, методологія.

Електронна адреса для листування з авторами:
yuliankonechnyi@gmail.com