UDC 615.9+582.929.4

INVESTIGATION OF ACUTE TOXICITY AND HEPAPROTECTIVE EFFECT OF EXTRACTS OF *NEPETA CATARIA* L.

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Key words: extract, *Nepeta cataria* L., acute toxicity, hepaprotective effect, medicinal plants material.

Actual problem of pharmacy is development of new medicines on the basis of herbal raw material. Searching herbs with sufficient raw materials, rational use of raw materials, implementation of wild herbs into culture and creation new medicines on their basis are important tasks of modern pharmacy. Recently, herbal products have gained attention as a major part of alternative medicine [1 – 3]. It is reported that a significant percentage of population depend on natural derived medicines for maintaining health and treatment of diseases [4]. Nowadays, discovery of new drug leads seems to focus on those of plant origin. Herbal drugs play a significant role in the regeneration of liver cells and acceleration of healing process and hence management of many liver disorders [4]. A valuable source of biologically active substances is *Nepeta cataria* L.

Liver is a large organ responsible for metabolism, detoxification, and protein synthesis [5, 6]. Drug-induced hepatotoxicity is one of the major causes of human mortality all over the world [7, 9 – 10].

Hepatobiliary diseases take a significant place in the clinic of internal diseases. Therefore, it is actual to research herbs, which have hepatoprotective effect. In alternative medicine *Nepeta cataria* L. has been used as a sedative medicine, for treatment of stomach and liver diseases, bronchitis and cold diseases for a long time [1 – 4]. Scientists determined that under the influence of phenolic substances, which are contained in *Nepeta* species, activity of free radical oxidation is depressed, which ensure their hepatoprotective effect [5, 6]. This motivates expediency of study of acute toxicity and hepatoprotective effect of dry extracts of *Nepeta cataria* L. herb.

Scientific research is a fragment of complex scientific research work of the Pharmacy Department of Ivano-Frankivsk National Medical University «Research of cultivated and wild medicinal plants of the Western region of Ukraine and development of technologies for their use for medical purposes» (Pharmacy Department of Ivano-Frankivsk National Medical University, state registration number 0118U003809).

Results of previous phytochemical investigations show that *Nepeta cataria* L. is a perspective source of valuable biologically active substances.

To use MPM rationally and to create new medicines ways of obtaining extracts based on *Nepeta cataria* L. herb have been previously developed and their pharmacological properties investigated.

Professor of the Department of Biological and Medical Chemistry with a course of physicolloidal and bionorganic chemistry Klymenko A.O. and Professor Gudyvok Y.S. of Pharmacology Department assisted in studying biologically activity of extracts of *Nepeta cataria* L. herb Experiment was done in the form of a simple pharmacological screening.

Experiment was conducted on white nonlinear mice and rats grown up in the vivarium of IFNMU, which were standardized according to physiological and biochemical indicators, and lived in the vivarium according to the requirements of sanitary-hygienic norms on a normal diet.

Biological effect on the animals was studied according to the International requirements (Guideline 86 / 609 / EU) and according to the «Methodical recommendations of keeping animals and working with them» [11].

All the material has been worked out with the method of variation statistics with the calculation of the average arithmetic and its standard error, authenticity reliability of dimensions compared has been assessed by the Student's criterion, level of probability has been accepted as $p \leq 0,05$ [12].

The aim of work was to study acute toxicity and hepatoprotective effect of dry extracts of *Nepeta cataria* L. herb.

The objects of investigation were dry extracts of *Nepeta cataria* L. herb. Studying of acute toxicity is a compulsory stage of researching new medicines, which allow assess danger of substances for health under the conditions of short-term effect

and determine class of toxicity and extent of therapeutic effect. Therefore, we have determined acute toxicity of extracts of *Nepeta cataria* L. herb at the first stage.

Studying of acute toxicity of extracts based on *Nepeta cataria* L. herb was delivered on the white nonlinear mice grown up in the vivarium of IFNMU. After intragastric injection of extracts of *Nepeta cataria* L. herb in a dose of 5000 mg/kg no animal has died.

To determine acute toxicity, the method of preclinical study of drugs harmlessness was used. The study of hepatoprotective activity of extracts of Nepeta cataria L. was carried out on the model of acute tetrachloromethane hepatitis. Hepatoprotector of domestic production – "Silibor" tablets were used as a comparison drug [8].

We conducted investigations on white rats-male which weigh 0.18 – 0.25 kg. Animals' liver was damaged by 50 % oil solution of tetrachlormethane, which was injected subcutaneously as 0,8 ml for 100 g of the animal weight during two days with the interval of 24 hours. The extracts investigated and the comparative drug «Sylibor» have been injected one hour before and in 2 hours after the injection of hepatotropic poison in a dose of 2.5 mg/0.1 kg of the animal weight [8, 13].

Rats have been decapitated (under etheric anesthesia) at the third day from the first moment of tetrachlormethane injection. We have made a conclusion about pharmacotherapeutical efficiency of extracts investigated based on the biochemical and functional indicators of liver, which have been determined in 24 hours after the last injection of tetrachlormethane.

Intensity of damage of liver cell membrane was assessed by the level of activity of transminase – Alanine transaminase (ALT), Aspartat transminase (AST). By the level of alkaline phosphatase we determine stagnant phenomenon in liver.

The rise in the ALT is usually accompanied by an elevation in the levels of AST, which play a vital role in the conversion of amino acids to keto acids [14]. In hepatotoxicity the transport function of liver cells is disturbed, causing leakage of plasma membrane [15], therefore resulting in leakage of these enzymes leading to an increase in their serum level. The increased level of ALT and AST in acetaminophen-

induced liver injury is an indicator of cellular leakage and loss of membrane integrity of liver cells [16].

The elevated serum level of alkaline phosphatase is due to its increased synthesis by bile canaliculi cells lining in response to the increased biliary pressure and cholestasis [17 – 19].

We have determined activity of transminases ALT and AST by an unified dynitrophenilhydrazive method of Raitman-Frenkel with the help of a standard set of reagents of «SIMKO Ltd». The point of this method is that as a result of reaming, which is taking place under the influence of enzymes (ALT and AST), pyruvic and oxalic acids are formed. When adding dynitrophenilhydrazine, the enzymatic process stops and hydrazones are formed, which give color in alkaline environment, the intensity of which is proportional to the number of formed accordingly pyruvic and oxalic acids. Colored hydrazones have maximum absorption with the length of wave 500 – 560 nm [17].

Activity of alkaline phosphatase has been determined by standard sets of reagents of «Filisit - Diagnostika» company. The principle of this method is the alkaline phosphatase splits phenylphosphate with a formation of phenol. The complex of phenol with N – aminophenazone forms red color, the intensity of which has been determined photometrically.

Results of investigation of acute toxicity of dry extracts of *Nepeta cataria* L. herb are shown in Table 1.

Table 1

Results of investigation of acute toxicity of extracts of *Nepeta cataria* L.

No	Name of substance investigated	Conditional marking	LD_{50}, mg/kg
1.	Water extract of *Nepeta cataria* L. herb	NCW	> 5000
2.	Water- alcohol extract of *Nepeta cataria* L. herb	NCS	> 5000

The animals were tidy, had a satisfactory appetite with good reaction to audio and light irritants, the urination and defecation processes were in the norm without any

interruption of breath and cramps. We haven't noticed any undesirable effects when injecting extracts, which were equivalent to 5000 mg/kg.

Investigation of acute toxicity (Table 1) shows that extracts of *Nepeta cataria* L. herb are practically non-toxic substances when making intragastric injection. Therefore, we can conclude that according to the classification of substances according to toxicity, Sidorov K. K. these phytosubstances can be classified as toxicity class V (low toxic compounds) [8].

Results of biochemical and hematological blood indicators are shown in Table 2. Table 2

Effect of extracts of *Nepeta cataria* L. herb on biochemical indicators of liver with acute toxic hepatitis

Object of investigation	Number of animals in the group	Erythrocytes, c/l	Leukocytes, g/l	Hemoglobin, g\l	AST, mcmol/h·mL	ALT, mcmol/ h·mL	Alkaline phosphatase, mc/cat/l
50% oil solution of CCl_4	6	6.18± 0.075	16.8± 0.37	101± 1.74	0.408± 0.021	0.86± 0.021	2.12± 0.009
Water extract of *Nepeta cataria* L. (NCW)	6	6.22± 0.13*	15.1± 0.44*	122± 1.90*	0.165± 0.0018*	0.213± 0.0016*	1.70± 0.021*
Water-alcohol extract of *Nepeta cataria* L. (NCA)	6	6.10± 0.08*	15.2± 0.31*	105± 0.78*	0.210± 0.0018*	0.336± 0.003*	1.92± 0.019*
Sylibor	6	6.30± 0.11	14.8± 0.35	118± 0.91	0.179± 0.0039	0.220± 0.0024	1.84± 0.042
Intact animals	8	6.40± 0.075	12,9± 0.27	134± 0.96	0.16± 0.010	0.19± 0.011	1.58± 0.015

Note* - authenticity of deviations regarding data of a control group ($p \leq 0,05$).

Results of investigations delivered (Table 2) show that extracts of *Nepeta cataria* L. herb on the model of reveal antioxidant and anticytolytic activity toxic damage of liver.

Simultaneous injection of hepatotropic poison and freeze-dried extracts of *Nepeta cataria* L. herb reduced the level of lipid peroxidation products (LOPS) and activity of ALT taking indicators shown to the level of intact animals.

Simultaneous injection of hepatotropic poison and investigated extracts investigated allowed determine a significant reduction of activity of AsAT, AlAT and dosage form with a clear tendency to the norm in the 2^{nd} and 3^{rd} groups of animals. Regarding indicators of peripheral blood, one should notice analogical regularity of change of investigated indicators.

Under the conditions of pathology, water-alcohol extract of *Nepeta cataria* L. herb reduced activity of markers of hepatocytes cytolysis – enzyme AlAT 2,5 times; AST – in 1,9 times.

The medicine showed inhibiting effect on activity of doses from, reducing activity of this enzyme in 3 times as compared to the control group of animals.

Results of investigations show that water- alcohol extract of *Nepeta cataria* L. herb on the model of reveals hepatoprotective activity acute toxic damage of liver.

As a result of study of acute toxicity and hepatoprotective effect of dry extracts of *Nepeta cataria* L. herb we can make a conclusion that:

1. A set of investigations as to studying acute toxicity of dry extracts of *Nepeta cataria* L. herb on mice allowed to determine the absence of toxic effect of medicines when making intragastric injections (LD_{50} >5000 mg/ kg). Therefore, we can conclude that according to the classification of substances according to toxicity, Sidorov K. K. these phytosubstances can be classified as toxicity class V (low toxic compounds).

2. Results of conducted investigations show that use of water- alcohol extract of *Nepeta cataria* L. herb revealed more intensive and efficient effect on hepatobiliary system as compared to water extract. Further on investigations have been conducted using water- alcohol extract.

Based on the results of our investigations, we were the first who suggested use of extracts of *Nepeta cataria* L. herb as a medical herbal raw material, which reveal hepatoprotective properties.

It is perspective to conduct further experimental investigation of extracts of *Nepeta cataria* L. herb as a medical plants material, which has sedative, antiphlogistic and antimicrobial properties.

Conflict of Interests. The authors declare that there is no conflict of interests regarding the publication of this paper.

ДОСЛІДЖЕННЯ ГОСТРОЇ ТОКСИЧНОСТІ ТА ГЕПАТОПРОТЕКТОРНОЇ ДІЇ ЕКСТРАКТІВ ТРАВИ *NEPETA CATARIA* L.

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Результати попередньо проведених фітохімічних досліджень вказують, що трава *Nepeta cataria* L. є перспективним джерелом цінних біологічно активних речовин. Метою роботи було вивчення гострої токсичності та гепатопротекторної дії сухих екстрактів трави *Nepeta cataria* L. Матеріали і методи: нами проведено вивчення гепатозахисної активності екстрактів *Nepeta cataria* L. на скринінговій моделі гострого тетрахлорметанового гепатиту [8], за методикою О.В. Стефанова. Як препарат порівняння був використаний гепатопротектор вітчизняного виробництва таблетки «Силібор» (Стефанов О.В., 2001).

Вивчено гостру токсичність та гепатопротекторну дію сухих екстрактів трави *Nepeta cataria* L. Встановлено, що екстракти трави *Nepeta cataria* L. є практично нетоксичними речовинами при внутрішньошлунковому введенні. Можна зробити висновок, що відповідно до класифікації речовин за токсичністю Сидорова К. К. дані фітосубстанції можна віднести до V класу токсичності (малотоксичні сполуки).

Результати досліджень свідчать, що водно-спиртовий екстракт трави *Nepeta cataria* L. при гострому токсичному ураженні печінки проявляє гепатопротекторну активність.

Ключові слова: екстракт, *Nepeta cataria* L., гостра токсичність, гепатопротекторна дія, лікарська рослинна сировина.

ИССЛЕДОВАНИЯ ОСТРОЙ ТОКСИЧНОСТИ И ГЕПАТОПРОТЕКТОРНОГО ДЕЙСТВИЯ ЭКСТРАКТОВ ТРАВЫ *NEPETA CATARIA* L.

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Результаты предварительно проведенных фитохимических исследований указывают, что трава *Nepeta cataria* L. является перспективным источником ценных биологически активных веществ. Целью работы было изучение острой токсичности и гепатопротекторного действия сухих экстрактов травы *Nepeta cataria* L. Материалы и методы: нами проведено изучение гепатозащитной активности екстрактов Nepeta cataria L. на скрининговой модели острого тетрахлорметанового гепатита [8] по методике А.В. Стефанова. В качестве препарата сравнения был использован гепатопротектор отечественного производства таблетки «Силибор» (Стефанов А.В., 2001).

Изучено острую токсичность и гепатопротекторное действие сухих экстрактов травы *Nepeta cataria* L. Установлено, что экстракты травы *Nepeta cataria* L. практически нетоксичные веществами при внутрижелудочном введении. Можно сделать вывод, что согласно классификации веществ по токсичности Сидорова К. К. данные фитосубстанции из листьев брусники можно отнести к V классу токсичности (малотоксичные соединения).

Результаты исследований свидетельствуют, что водно-спиртовой экстракт травы *Nepeta cataria* L. при остром токсическом поражении печени проявляет гепатопротекторное активность.

Ключевые слова: экстракт, *Nepeta cataria* L., острая токсичность, гепатопротекторное действие, лекарственное растительное сырье.

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