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RESEARCH OF THE HEPATOPROTECTIVE ACTIVITY OF SWEET WOODRUFF DRY EXTRACT

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Sweet woodruff (Asperula odorata L.) of Rubiaceae Juss. family is not officinal plant, which is used in folk medicine as a sedative, diuretic and choleric agent. The hepatoprotective activity of a dry extract of sweet woodruff herb has been determined experimentally on the model of the acute toxic liver damage in rats. It has been found that the extract of sweet woodruff in the dose of 25 mg/kg reduces the intensity of lipid peroxidation, normalizes biochemical indicators of the animal blood and the liver homogenate to the level of intact animals, decreases toxic effects of tetrachlormethane. Moreover, the activity of ALT and AST in the blood serum decreases by 2.2 and 2.1 times, respectively; the cholinesterase activity increases by 13%, the level of TBA-reagents in the blood serum and the liver tissue decreases by 1.3 and 1.6 times, respectively, compared to the control group of animals. To confirm the results of the research the study of the histological structure of the liver of the experimental animals has been carried out. While studying the histology of the liver in treated animals the degenerative changes and the phenomenon of structural degradation and signs of severe hemodynamic disorders have not been detected. By its hepatoprotective action the sweet woodruff extract is practically not inferior to the reference drug Silibor.

Treatment of chronic diseases of the liver and biliary tract requires the use of medicines for a long period of time. To normalize metabolic processes in the liver and the structural and functional integrity of the cell membranes of hepatocytes hepatoprotective agents with the antioxidant, membrane stabilizing, detoxification, choleric and immunomodulatory effects are widely used [8].

Important features of herbal drugs are their mild effect on the human body, a wide range of therapeutic effects and low toxicity. Therefore, it is important to find new types of herbal drugs that exhibit hepatoprotective and choleric properties.

From the analysis of primary scientific sources the herb of sweet woodruff is known to be used as a diuretic, diaphoretic, sedative and cholagogue agent.

We have previously studied amino acids, fatty acids and organic components of the essential oil of sweet woodruff's herb [6, 7, 9].

The aim of this work was to study the hepatoprotective activity of the dry extract of sweet woodruff herb on the model of the acute tetrachloromethane-induced hepatitis.

Materials and Methods

The object of our study was the air-dried herb of sweet woodruff (*Asperula odorata* L.) harvested at the flowering stage in the summer of 2012 in the Kharkiv region. The dry extract was obtained by 3-fold extraction of the raw material with 70% ethanol at the ratio of 1:3. The liquid extracts were evaporated under vacuum in order to remove the extractant and obtain a dry residue. The yield was 7.6%. The main active substances of the extract were hydroxycinnamic acids and flavonoids. Their content was determined by the spectrophotometric method and was 7.57% of hydroxycinnamic acids (equivalent to chlorogenic acid) and 3.69% of flavonoids (equivalent to rutin).

The research of the hepatoprotective activity was carried out on the model of the acute tetrachloromethane-induced hepatitis in white male rats weighing 0.18-0.25 kg. They were divided into 4 groups of 6 animals each: the first group – animals that were injected subcutaneously with the sweet woodruff extract in the dose of 25 mg/kg, the second group – animals treated with the reference drug Silibor in the dose of 25 mg/kg, the third group – the control group consisting of untreated animals and the fourth group – the intact animals. The liver disease was modeled by a single subcutaneous injection of 50% oil solution of tetrachloromethane in the dose of 0.8 ml per 0.1 kg of the animal to the first three groups for 2 days with the interval of 24 hours. The dry extract studied and the reference drug were injected subcutaneously to the animals of the first and second groups for 1 hour before and in 2 hours after the hepatotropic poisoning [1, 5].

Rats were decapitated on the third day after the first injection of tetrachloromethane. The conclusion of the pharmacotherapeutic efficiency of the extracts studied was based on the biochemical and

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Table

The effect of the sweet woodruff dry extract on the biochemical indices of the blood and the state of the liver in acute hepatitis

Biochemical and hematological indices	Objects of the research			
	Sweet woodruff dry extract	Silibor	50% oil solution of CCl ₄	Intact animals
Blood serum				
ALT, mcmol/h.ml	0.61 ± 0.015*	0.27 ± 0.008*	1.36 ± 0.04*	0.24 ± 0.015*
AST, mcmol/h.ml	0.52 ± 0.014*	0.23 ± 0.008*	1.08 ± 0.04*	0.20 ± 0.015*
CE, μkat/L	66.5 ± 0.76*	72.3 ± 1.45*	57.8 ± 1.54*	84.5 ± 1.89
ALP, μkat/L	1.97 ± 0.13*	1.85 ± 0.07*	2.08 ± 0.13*	1.60 ± 0.08
TBA-reagents, nmol/ml	4.53 ± 0.04*	3.86 ± 0.05*	5.84 ± 0.02*	3.52 ± 0.07
Liver tissue homogenate				
TBA-reagents, mkmol/g	38.2 ± 0.76*	33.5 ± 0.87*	62.2 ± 1.51*	26.2 ± 0.63

Note: * – Reliability of deviation comparing to the control group of animals ($p \leq 0.05$).

functional indices of the liver and serum determined in 24 hours after the last tetrachlormethane injection.

To assess the primary hepatoprotective effect the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholinesterase (CE) and alkaline phosphatase (ALP) was determined in the blood serum. The rate of formation and the content of lipid peroxidation products in the liver tissue – TBA-reagents, the specific markers of cytolysis, were also found.

Determination of the activity of ALT and AST was performed by the standardized dinitrophenylhydrazine method of Reitman – Frankel using standard reagents of “SIMKO, Ltd.” company [3].

Determinations of the cholinesterase activity showing the state of the protein synthesis function in the serum, as well as the activity of alkaline phosphatase indicating a dysfunction of the enzyme secretion by hepatocytes in the bile, and development of congestion in the liver were performed by the spectrophotometric method using “Lachema” standard diagnostic kits (Czech Republic) [3].

The level of lipid peroxidation products – TBA-reagents was evaluated spectrophotometrically by the reaction of 2-tiobarbituric

acid according to the method of E.N.Korobeinikova using biochemical kits of the Ukrainian producer (a reagent kit of “Filisit – Diagnostic” company, Ukraine) [2, 4]. The study of microslides was performed under a Mikros 400 microscope. Photomicrography was carried out using a Nikon Col Pix 4500 digital camera. Photos were processed on a Pentium 4 GH computer using the Nikon View 5 programme.

Statistical analysis of the results was performed using the methods of variation statistics. Reliability of intergroup differences was determined using Student’s t-test.

Results and Discussion

One of the criterion of the hepatoprotective effect of the extract under study is the rate of animals survival [1]. Mortality in the control group was 16.7%, in all other groups the animals were alive by the end of the experiment.

The research results (Table) indicate that the toxic effects of tetrachlormethane on the liver of untreated animals was characterized with development of cytolysis of hepatocytes as evidenced by a significant increase in the enzyme activity of ALT and AST by 5.7 and 5.4 times, respectively, compared to the indices of intact animals.

At the same time increase of the TBA-reagents content in both the serum and the liver tissue homogenate in animals by 1.65 and 2.37 times, respectively, compared to those of intact animals was observed. The level of the cholinesterase activity in the blood serum of animals in the control group decreased by 31.6%, the level of the alkaline phosphatase activity increased by 30.0% compared to the group of intact animals. The results obtained indicate the presence of the oxidative stress in animals.

Introduction of the sweet woodruff dry extract had positive effects on the liver and improved the blood biochemical indices: the activity of ALT and AST in the serum decreased by 2.2 and 2.1 times, respectively; the cholinesterase activity increased by 13%, the level of TBA-reagents in the blood serum and the liver tissue decreased by 1.3 and 1.6 times, respectively, compared to the control group of animals.

To confirm the results of the study, the research of histological structure of the liver of experimental animals has been carried out.

While studying the histology of the liver in treated animals the degenerative changes and the phenomenon of structural degradation and signs of severe hemody-

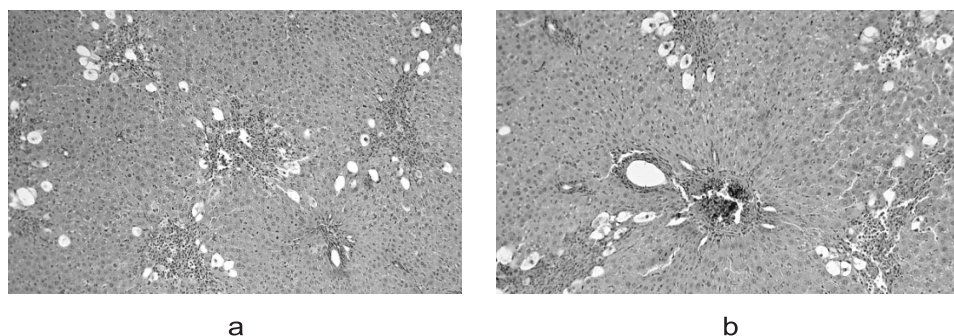


Fig. 1. The liver of the experimental animals: a) hepatocytes with full-blown vacuolar degeneration and preserved nucleus. Approximation: ocular 10, objective 10; b) in perysinusoidal spaces the focal accumulation of mononuclear cells. Approximation: ocular 10, objective 20. Coloured by hematoxylin and eosin

namic disorders have not been detected.

The results of the morphological studies of the internal organs of animals are presented in Fig. 1.

While studying the histological preparations of the first group of animals (Fig. 1a) it was found that the microscopic lobular structure of the liver was preserved. At the periphery of the particles along the portal tracts there were individual hepatocytes with full-blown vacuolar degeneration and preserved nucleus. The parenchyma hepatocytes were normal, somewhere increased in size, their nuc-

lei had a clear contour with a distinct nucleolus. The central veins were plethoric. Sinusoidal capillaries were irregularly plethoric. The ducts were expanded. The wall of the bile duct was represented by a single layer cubic and the cylindrical epithelium. There were focal accumulations of mononuclear cells in the stroma of portal tracts and around large vessels in the perysinusoidal spaces (Fig. 1b).

The results of pathomorphological studies of the liver of the experimental animals indicate that changes in the internal organs are comparable with intact animals.

CONCLUSIONS

The sweet woodruff dry extract in the dose of 25 mg/kg on the model of the tetrachlormethane-induced acute toxic liver damage in rats has shown the hepatoprotective effect normalizing biochemical indicators of the animal blood and the liver homogenate to the level of intact animals. This extract reduces the intensity of lipid peroxidation and decreases toxic effects of tetrachlormethane providing the hepatoprotective effect.

By its hepatoprotective action the sweet woodruff extract is practically not inferior to the reference drug Silibor.

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ДОСЛІДЖЕННЯ ГЕПАТОПРОТЕКТОРНОЇ АКТИВНОСТІ СУХОГО ЕКСТРАКТУ ТРАВИ МАРЕНКИ ЗАПАШНОЇ**Н.С.Юрченко, Т.В.Ільїна, А.М.Ковальова, А.О.Клименко*, В.М.Іваночко*****Національний фармацевтичний університет, Івано-Франківський національний медичний університет****Ключові слова: маренка запашна; гепатопротекторна активність; сухий екстракт*

*Маренка запашна *Asperula odorata* L. родини маренові *Rubiaceae* Juss. – неофіційна рослина, яка в народній медицині застосовується як седативний, сечогінний, жовчогінний засіб. Експериментально на моделі гострого токсичного ураження тетрахлорметаном печінки щурів встановлено гепатопротекторну активність сухого екстракту трави маренки запашної. Виявлено, що екстракт маренки запашної в дозі 25 мг/кг знижує інтенсивність процесу перекисного окиснення ліпідів у піддослідних тварин, доводить біохімічні показники крові та гомогенату печінки до рівня інтактних тварин, зменшуючи токсичну дію тетрахлорметану. При цьому активність АЛАТ та АсАТ в сироватці крові знижується в 2,2 та 2,1 рази відповідно; активність холінестерази зростає на 13%; рівень ТБК-реактивності у сироватці крові та тканині печінки знижується в 1,3 та 1,6 рази відповідно в порівнянні з контрольною групою тварин. Для підтвердження результатів проведено вивчення гістологічної будови печінки дослідних тварин. При вивченні гістологічної картини печінки у лікованих тварин досліджуваної групи дистрофічні зміни та явища деструкції структурної організації елементів органу, ознаки виражених розладів гемодинаміки виявлені не були. За ступенем гепатопротекторної дії екстракт маренки запашної практично не поступається препарату порівняння «Силибору».*

ИССЛЕДОВАНИЕ ГЕПАТОПРОТЕКТОРНОЙ АКТИВНОСТИ СУХОГО ЭКСТРАКТА ТРАВЫ ЯСМЕННОКА ДУШИСТОГО**Н.С.Юрченко, Т.В.Ильина, А.М.Ковалева, А.О.Клименко*, В.М.Иваночко*****Национальный фармацевтический университет, Ивано-Франковский национальный медицинский университет****Ключевые слова: ясменник душистый; гепатопротекторная активность; сухой экстракт*

*Ясменник душистый *Asperula odorata* L. семейства мареновые *Rubiaceae* Juss. – неофициальное растение, которое в народной медицине используется как седативное, мочегонное, желчегонное средство. Экспериментально на модели острого токсического поражения тетрахлорметаном печени крыс установлено гепатопротекторную активность сухого экстракта травы ясменника душистого. Виявлено, что экстракт ясменника душистого в дозе 25 мг/кг снижает интенсивность процесса перекисного окисления липидов у животных, доводит биохимические показатели крови и гомогената печени до уровня интактных животных, снижая токсическое действие тетрахлорметана. При этом активность АЛАТ и АсАТ в сыворотке крови снижается в 2,2 и 2,1 раза соответственно; активность холинэстеразы возрастает на 13%; уровень ТБК-реактивов в сыворотке крови и ткани печени снижается в 1,3 и 1,6 раза соответственно по сравнению с контрольной группой животных. Для подтверждения результатов проведено изучение гистологического строения печени исследуемых животных. При исследовании гистологической картины печени у леченых животных исследуемой группы дистрофические изменения и явления деструкции структурной организации элементов органа, признаков выраженных расстройств гемодинамики не были выявлены. По степени гепатопротекторного действия экстракт ясменника душистого практически не уступает препарату сравнения «Силибору».*

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