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Antioxidant properties of extracts of aerial part of *Bupleurum aureum*, hill-growing saltwort herb, *Fumaria schleicheri* and *Cynara scolymus in vitro* and *in vivo*

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ABSTRACT

The aim of this study was to investigate the potential antioxidative effects of herbal extracts of aerial part of Bupleurum Aureum, Hill-Growing Saltwort Herb, Fumaria Schleicheri and Cynara scolymus on carbon tetrachloride-induced oxidative stress in vitro and in vivo. It was observed, that administration of plant extracts to rats significantly improve the oxidant status in blood serum and in the cytosol of rat liver. It was found that all explored herbal extracts have antioxidative properties.

Key words: pharmacological screening, medicinal herbs, antioxidative activity.

INTRODUCTION

The development of novel plant-derived natural products and their analogs for the prevention and treatment of oxidative stress is an actual problem of modern medicine and pharmacy. It is known that many of stressors such as emotional stress, xenobiotics poisoning, acute alcoholism, essential microelements injection into organism in volumes which exceed physiological rates, lead to imbalance in the system prooxidants-antioxidants, and oxidative stress development [2,3]. It was recorded that such medical plants as Bupleurum aureum, Hill-Growing Saltwort Herb, Fumaria Schleicheri and Cynara scolymus have antioxidative properties [4,5,6-8].

Bupleurum aureum is Apiaceae herb used in Chinese and Japanese medicine already 2 thousand years ago. It protects liver from any kind of poisoning, damage effect of carbohydrates, petrochemical plants emissions, and protects also from consequences of radioactive radiation**.** There are saponins, essential oil, alkaloids, tannins, alcohol, vitamin C, carotin, flavonoids, rutin in its structure. From an alternative medicine it is known that Bupleurum liqueur changes chemical compound of bile, increases volume of bile acides, bilirubin and cholesterol. It shows immunomodulatory, hepatoprotective, anti-tumor and anti-viral activities, general health-improving effect on organism, promotes metabolic process improvement [9-12].

Salsola collina rates as herbaceous and frutescent plants of Chenopodiaceae row. Salsola collina is used in Chinese and Tibetan medicine from long ago, though in current medicine it was recollected recently. Hill-growing Saltwort herb is a strong hepatoprotector which normalizes function and metabolism of liver parenchyma, accelerates regeneration and standardizes functional activity of hepatocytes, improves oxidizing-reductive processes in liver [13]. High potassium causes prevention and treatment of ischaemic heart disease. Hill-growing Saltwort herb has an insulin-analogous effect, decreases blood sugar level. It consists of unique set of microelements including Silicon, Copper needed for hematopoietic system [14]. This herb has significant hepatoprotective effect while toxic influence of poisons on liver, that cause hepatitis, medical products, alcohol and other damaging factors, advantangeously influences on metabolic disturbance, liver's function and structure, prevents necrosis development, increases antitoxic function of liver. Its composition includes: betaine, sterines (have lipotropic, hepatoprotective, cholepoietic and cholagogus effects); alkaloids (show hepatoprotective effect and remove spasms of Smooth muscle); flavonoids (tricine, isorhamnetin, quercetin, rutin) – antioxidants; tokopherols – antioxidants; gamma-linoic acid – participates in prostaglandins' synthesis and normalizes biochemical blood indices; micro- and macroelements (Potassium, Silicon, Ferum, Zinc, Magnesium, Phosphorus) – improve mineral metabolism; aminoacids – improve protein metabolism; polysaccharides (mannans, inulin) – improve intestine microflora, reduce blood sugar level [15-18].

Healing properties of Cynara scolymus L. from Asteraceae, are known from a long time ago. In the days of Renaissance and in the middle of century it was applied as cholagogue, antireumatic, diaphoretic and diuretic preparation. Medicines based on this herb were used to whet appetite and for heart diseases. Current researches prove that extracts show its cholagogue properties, raise tone and evacuation of gallbladder contents, and also exert severe hepatoprotective activity due to antioxidative and hypocholesteremian effects. Have antimicrobial properties against different types of pathogenic bacterium, yeast bacillus and mycotic flora [19-21]. Most of pharmacological effects of artichoke are shown by means of high level of polyphenol antioxidants. An artichoke composition consists also of hydroxycinnamic acids, specific carbohydrates (inulin), a great volume of folic acid, magnesium, phosphorus. High volume of essential macro- and microelements have been identified [22,23].

Composition of Fumaria Schleicheri Soy-Wiilem. which belongs to Fumariaceae DC, includes alcaloids (protopine, fumaridine, fumarinine, fumaritine, M-methylhydrostein, M-methylhydristin, fumarion, oxyhydrostenin, bicuculline, stylopine, fumschleicherin), vitamines C, K, choline, tannins, flavonoids, lipids. Thanks to mentioned composition, Fumaria Schleicheri Soy-Wiilem. is applied as hypotensive preparation, for cardiovascular pathologies, stomack and lung diseases as antioxidative, wound healing and hemostatic drug. Fumaria Schleicheri Soy-Wiilem. has a capacity to clean blood through bile outflow regulation (stimulation while insufficient bile production and easing while exceed bile production), and due to all above eliminates chronic constipation, that, in turn, is a basic condition for chronic skin diseases treatment [24-26].

It is known, that antioxidative properties of polyphenol compounds are supposed to connect with their capacity to be trap for active metabolites of oxid, associate metal ions, which are lopid peroxidation inductors and inhibit activity of set of redox-sensitive transcription factors, NFkD (nuclear factor kappa B), AP-1 (activator protein 1) and prooxidative ferments particularly.

Thus, analysis of chemical composition properties of examined plants, based on study of literature data (existence of considerable amount of polyphenols, polyunsaturated fatty acids, tocopherols), became foundation for study on their antioxidizing and antioxidative properties.

EXPERIMENTAL SECTION

Antioxidizing properties of extracts in vitro have been studied on samples of spontaneous and ascorbate-induced Lipid peroxidation in rat liver homogenate [27]. Numbers of extracts added to the incubative environment, have been calculated on basis of dose, that were more effective in prior researches (0,1 mg/g of liver). As comparative preparation α-tokopherol were used in dosage of 50 мg/kg, because it is a vigorous lipophilic antioxidant [27,28].

Estimation of antioxidative activity of herbal extracts was carried out under conditions of rats' acute experimental hepatitis, caused by tetrachlormethane. In the present work the outbread rats-females were examined in wage of 180-200 g. CCL_4 was injected once intragastrically as 50 % oil solution of tetrachlormethane in dosage of 10 ml/kg. [27].One week before tetrachlormethane injection the animals were daily injected once per day into stomach by examined extracts in dosage of 2 mg/kg and 5 mg/kg and immediately after CCL_4 injection (for medical and preventive purpose). For comparison silibor was chosen in dosage of ED_{30} – 25 mg/kg and quercetine – in dosage of 5 mg/kg. The examined animals were divided into 8 experimental groups of 10 animals in each one:

- 1 group – intact animals;
- 2 group – control animals with experimental hepatitis;
- 3 group – animals that have taken extract of Bupleurum Aureum in dosage of 5 mg/kg;
- 4 group – animals that have taken extract of hill-growing Saltwort herb in dosage of 5 mg/kg;
- 5 group – animals, that have taken extract of Fumaria Schleicheri Soy-Wiilem. in dosage of 2 mg/kg;
- 6 group – animals, that have taken extract of Cynara scolymus in dosage of 5 mg/kg;
- 7 group – animals, that have taken reference drug quercetine in dosage of 5 mg/kg;
- 8 group – animals, that have taken reference drug silibor in dosage of 25 mg/kg.

Then animals were taken out of the experiment through euthanasia, liver was removed and blood was sampled for biochemical study. Liver was perfused by cold environment of discharge and homogenize in Potter-type apparatus on ice. Estimation of livers state was carried out on basis of following characteristics: animals' survival, liver's mass coefficient (LMC); in blood serum: processes of hepatocytes' destruction – according to activity of marker enzyme of alanine aminotransferase cytolysis (AAC). In liver's homogenate an intensity of lipid peroxidation processes was defined by level of thiobarbituric acid – active products; state of antioxidative system of animals' organism – by content of renewed glutathione (RG). Influence of preparations on biochemical indices of blood was identified according to standard methods [28,29]. Animals were managed in standard conditions of vivarium of NUPh Central scientific-research laboratory. Research was carried out in accordance with national «General ethical principles for experiments on animals» (Ukraine, 2001), that meet provisions of "European convention on Protection of spine animals", that are applied for experimental and other scientific purposes (Strasbourg, 1985). Procedures accompanied with paint (surgical attack, euthanasia, devisceration) were carried out under anesthesia (thiopental sodium in dosage of 80 mg/kg abdominally). Qualitative data were processed by means of program for statistical processing of data StatPlus 2009.

RESULTS AND DISCUSSION

According to research results (Fig. 1.) in incubation of liver's homogenate in buffered solution at temperature 37°C sizable accumulation of thiobarbituric acid-reactants was shown, that indicates intensive progress of the lipid peroxidation processes. Therewith, growth of TBA-reactants content becomes appeared in the first 20 minutes of incubation. After 20 minutes content of thiobarbituric acid-reactants did not change, that evidently connects with exhaustion of lipid peroxidation substrates. Storage of thiobarbituric acid-reactants was more evidential after ascorbate addition in incubative environment as high-powered inductor of nonenzymatic lipid peroxidation. Thus, velocity of thiobarbituric acid-reactants storage in spontaneous LP during first 20 minutes of incubation equals 0,45 nM/l per 1 minute, in ascorbate-inductive LP – 0,55 nM/l per a minute.

The obtained data indicate capacity of experimental herbal extracts to block lipid peroxidation processes already for the first minutes after beginning of incubation. Evidently, it's connects with presence of polyphenoles which are part of composition of experimental herbal extracts. It is known that polyphenoles exactly are capable to couple active oxid metabolites, that are lipid peroxidation inductors at early stages [2].

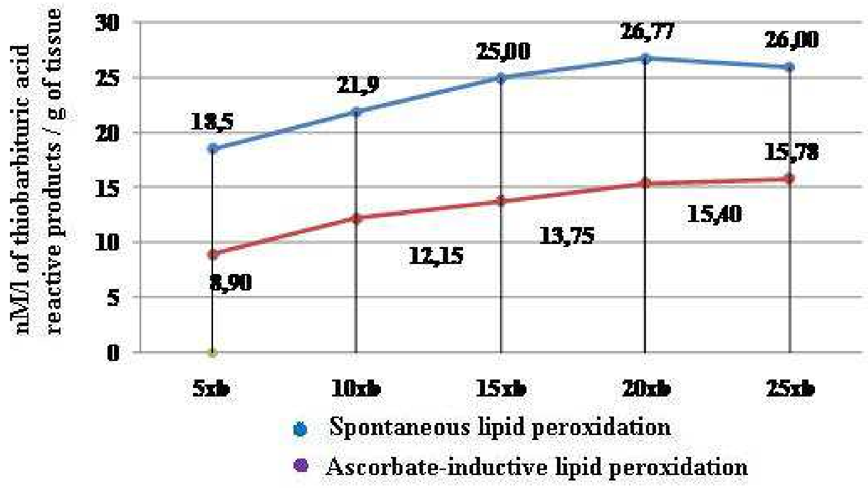

Fig.1. Dynamic of thiobarbituric acid-reactants storage in incubation of rats liver homogenate at t 37°C under lack of inductors and ascorbate existence

On addition of extracts of Bupleurum Aureum, hill-growing Saltwort herb, Fumaria Schleicheri and Cynara Scolymus to incubative environment, content of thiobarbituric acid-reactants in 5 minutes from the beginning of incubation comparing to control was lower 3,14; 2,51; 1,7 and 1,85 times respectively (Fig. 2) for spontaneous lipid peroxidation, and 3,8; 3,3; 2,6 and 3,0 times lower for ascorbateinductive lipid peroxidation (Fig. 3). Meanwhile, increase of level of thiobarbituric acid-reactants after 5 minutes of incubation was not observed both on condition of spontaneousandascorbate-inductivelipid peroxidations.

Capacity of experimental extracts to inhibited ascorbate-inductive lipid peroxidation may be connected with coupling of Ferrum ions by poliphenoles, needed for induction of lipid peroxidation by ascorbate. On addition of extracts of Bupleurum Aureum and hill-growing Saltwort herb to incubative environment we have registered less expressed TBB-reactants comparing to trials, to which extracts of Fumaria Schleicheri and Cynara Scolymus have been added. On addition of α-tocopherol to incubative environment TBB-reactants storage is also less evidential in comparison with control group of animals, but more expressed compared to extracts of Bupleurum Aureum and hill-growing

Saltwort herb in dosage of 0,5 mg/g of liver. So, in spontaneous lipid peroxidation content of TBB-reactants during terms of experiment on addition of α-tocopherol to incubative environment was in average 1,5 and 1,2 times higher, and in ascorbate-inductive lipid peroxidation - 1,5 and 1,3 times higher compared to trials, to which extracts of Bupleurum Aureum and hill-growing Saltwort herb have been added.

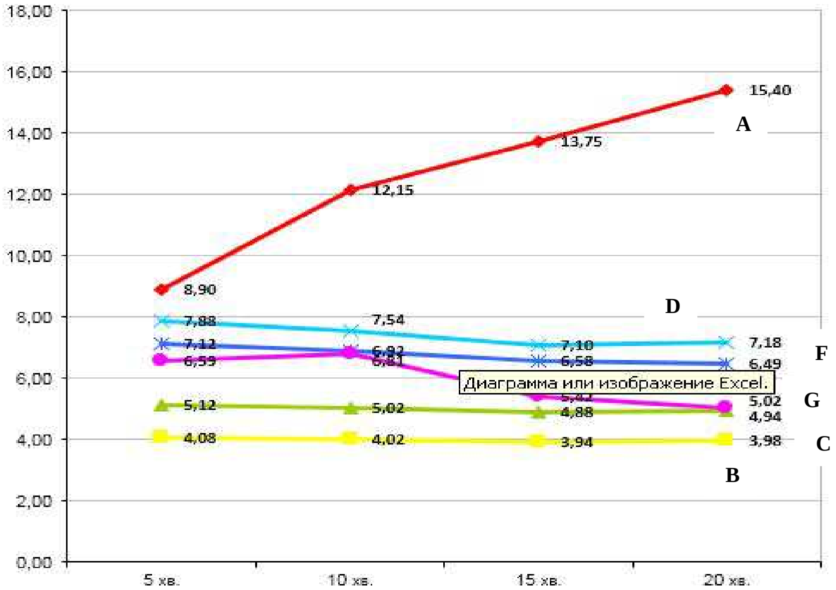

Fig. 2 Influence of extracts of Bupleurum Aureum, hill-growing Saltwort herb, Fumaria Schleicheri and Cynara Scolymus and α-tocopherol on progress of spontaneous lipid peroxidation in incubation of rats liver homogenate at t 37°C, n=5 (A – control pathology; B – extract of Bupleurum Aureum; C – extract of hill-growing Saltwort herb; D – extract of Fumaria Schleicheri; F – extract of Cynara Scolymus; G – α-tocopherol)

*Notes: * – discrepancy authentic concerning control pathology, p≤0,05;*
*** – discrepancy authentic concerning α- tocopherol, p≤0,05*

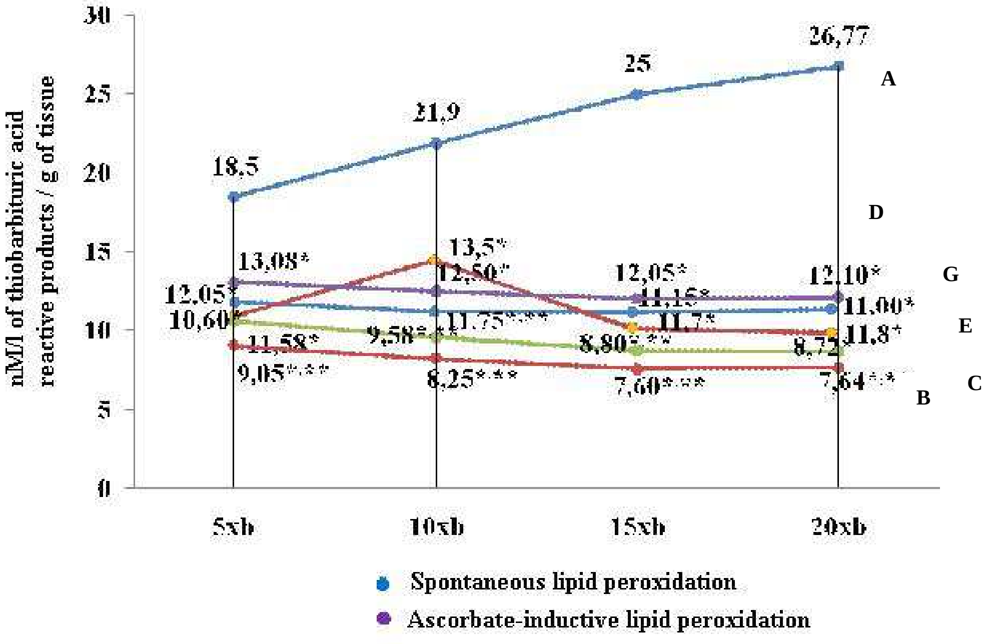

Fig. 3 Influence of extracts of Bupleurum Aureum, hill-growing Saltwort herb, Fumaria Schleicheri, Cynara Scolymus and α-tocopherol on progress of ascorbate-inductive lipid peroxidation in incubation of rats liver homogenate at t 37°C in presence of ascorbate, n=5 (A – control pathology; B – extract of Bupleurum Aureum; C – extract of hill-growing Saltwort herb; D – extract of Fumaria Schleicheri; F – extract of Cynara Scolymus; G – α-tocopherol)

*Notes: * – discrepancy authentic concerning control pathology, p≤0,05;*
*** – discrepancy authentic concerning α-tocopherol, p≤0,05*

Thereby, obtained data define that all herbal extracts may block both, spontaneous and ascorbate-inductive activation of lipid peroxidation processes in vitro, that proves their antioxidizing activity. There was founded that extracts of Bupleurum Aureum and hill-growing Saltwort herb have the most expressed activity.

While further set of trials we have studied antioxidative properties of herbal extracts on a sample of rats' acute tetrachlormethane hepatitis. Liver injury by tetrachlormethane is a classical model of so-called free-radical pathologies, which is more often used for study on antioxidative properties of pharmacologically active substances. High hepatoprotectiveness of tetrachlormethane is connected with its high solubility in lipids and storage in hydrophobic sphere of biomembranes and membrane's structural damage by activation of lipid peroxidation processes [1,30].

Results of researches, presented in table 1, testify that poisoning by tetrachlormethane for animals from control group was accompanied with development of ulcerative syndrome of peroxidation, that appeared by growth of content of TBB-active compounds in liver tissue in 2,6 times, and level decreasing of renewed glutathione in 1,44 times. At the same time, activity of AAC in blood serum raised in 3,4 times which specifies on development of syndrome of cytolysis. Mortality among animals from group of control pathology was 30%.

Table 1. Influence of extracts of Bupleurum Aureum, hill-growing Saltwort herb, Fumaria Schleicheri and Cinara Scolymus on functional-biochemical properties of blood serum under acute tetrachlormethane hepatitis

Terms of experiment	n	Animals mortality	TBB-AP, micromole/g	RH, standard unit	AAC, mm/g*l
Intact control	10	0	33,69±4,40	33,91±3,10	0,42±0,04
Control Pathology	10	3	88,94±9,57*	23,52±2,60*	1,43±0,10*
Extract of Bupleurum Aureum	10	0	45,23±7,96*/**	30,27±2,04**	0,65±0,16*/**
Extract of hill-growing Saltwort herb	10	0	48,44±8,56*/**	30,24±2,31**	0,72±0,14*/**
Extract of Fumaria Schleicheri	10	0	65,70±6,33*/**	29,08±1,93**	0,7±0,12*/**
Extract of Cynara Scolymus	10	0	54,83±7,94*/**	29,67±2,44**	0,78±0,11*/**
Quercetine, 5 mg/kg	10	0	55,67±6,12*/**	29,11±2,24**	0,83±0,10*/**
Silibor, 25 mg/kg	10	1	54,49±5,73*/**	30,81±2,44**	0,79±0,08*/**

*Notes: * – discrepancy authentic concerning intact control, p≤0,05;*
*** – discrepancy authentic concerning control pathology, p≤0,05;*
n – numbers of experimental animals in group.

Preventive and medical injection of extracts of Bupleurum Aureum, hill-growing Saltwort herb, Fumaria Schleicheri and Cynara Scolymus was accompanied with distress of peroxidative destructive processes and decreasing of content of TBB-active compounds in liver tissue up to 34,5%. Reference-preparations Silibor and Quercetine created pretty more expressive influence on lipid peroxidation progress and diminished level of TBB-active compounds up to 49,1%; 45,5%; 26,1% and 38,4%, respectively. Rather more expressive influence on the lipid peroxidation progress comparing to the reference-preparations Silibor and Quercetine showed extracts of Bupleurum Aureum and hill-growing Saltwort herb, which caused decreasing of TBB-active compounds level to 49,1% and 45,5% (p≤0,05), corresponding to groups of control pathology. The least antioxidative activity have shown extracts of Fumaria Schleicheri by reducing TBB-reaktantants to 26,1%.

The analysed preparations also positively affected on state of the own antioxidative system, which is proved by growth of the level of renewed glutathione in all experimental groups practically to the level of intact control.

Among studied preparations the extracts of Bupleurum Aureum, hill-growing Saltwort herb and Cynara Scolymus by diminishing the AAC activity to 54,5%; 49,7% and 45,5%. The examined extracts of Bupleurum Aureum and hill-growing Saltwort herb have found advantage compared to referent preparations due to their anticytolytic activity. All rats in groups which have been given examined extracts, survived. After injection of Cylibor nine animals survived (90%).

Hereby, results of conducted researches testify that extracts of Bupleurum Aureum, hill-growing Saltwort herb, Fumaria Schleicheri and Cynara Scolymus show evidential antioxidative properties and reduce expressiveness of cytodestructive processes, conditioned by activation of lipid peroxidation. On expressiveness of medical effect under acute tetrachloromethane hepatitis only Fumaria Schleicheri extract is inferior to reference-preparations silibor and quercetine on anticytolytic and antioxidative activities.

CONCLUSION

1. Herbal extracts of Bupleurum Aureum, hill-growing Saltwort herb, Fumaria Schleicheri and Cynara Scolymus may effectively block both, spontaneous and ascorbate-inductive activation of processes of lipid peroxidation in

vitro, that is proved by their antioxidizing activity. There was founded that extracts of Bupleurum Aureum and hill-growing Saltwort herb have the most expressed activity.

2. On the model of acute tetrachloromethane hepatitis it was defined that examined herbal extracts show antioxidative properties of different intensity, depress progress of peroxide destructive processes, improve greatly state of own antioxidative system and reduce expressiveness of cytolytic syndrome.

3. On the model of acute tetrachloromethane hepatitis it was established that extracts of an aerial part of Bupleurum Aureum and Hill-Growing Saltwort herb in dosage of 5 mg/kg show expressed antioxidative properties with depress of peroxide destructive processes progress, substantially improve state of own antioxidative system, that normalization of renewed glutathione proves, and reduce expressiveness of cytolytic syndrome. On antioxidative and anticytolytic activities, in conditions of an acute oxidative stress these extracts express are inferior to reference-preparations silibor and quercetine.

4. High effectiveness and wide spectrum of pharmacological properties of extracts of aerial part of Bupleurum Aureum and Hill-Growing Saltwort herb can be described by their chemical composition that consists of phenol-type compounds, phytosterols, saikosaponins, aminoacids, tochopherols, unsaturated fatty acids and large amount of macro- and microelements.

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