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THE ELEMENTAL COMPOSITION OF DRY EXTRACTS FROM *GALIUM VERUM* L. HERB

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Introduction. *Galium verum* L. (*Rubiaceae* Juss.) is an ethnomedicinal plant of a wide spectrum of biologically active substances (BAS) and pharmacological effects. We previously reported on a hepatoprotective effect of dry extracts (extract **I** and extract **II**) from *G. verum* herb on carbon tetrachloride-induced injury in rats [2]. The hepatoprotective effect of extract **II** was comparable with the hepatoprotective activity of the reference drug Silibor and was more pronounced than that of the extract **I.** Based on studied biochemical parameters, the possible mechanism of the hepatoprotective activity of the extracts could be explained through a cytolysis inhibition and a reduction of oxidative stress. The results obtained highlighted the need for in-depth comparative analysis of BAS of the dry extracts.

Experimental results show beneficial effects of macro and trace elements in maintaining the normalization of functional state and histoarchitecture of the liver in different animal models of liver injury [3-6, 8-9].

An aim of this work was to study the elemental composition of dry extracts from *G. verum* herb (extract **I** and extract **II**) in order to justify differences in established levels of hepatoprotective activity, and to calculate plant to extract transfer factors for macro and trace elements taking into account our previously obtained data on elemental composition of *G. verum* herb [1].

Materials and methods. The objects of this study were two dry extracts from *G. verum* herb: extract **I** was obtained from 70% ethanolic extract purified from lipophilic compounds, and extract **II** – from the aqueous extract purified from polysaccharides. Study of the elemental composition was carried out using atomic emission spectrophotometry on the base of DNU "STC" Institute for Single Crystals" of NAS of Ukraine according to the previously described procedure [1].

Results and their discussion. In the dry extracts 14 elements were identified and quantified (Table 1), of which 6 macro (K, Na, Ca, Mg, Si and P) and 8 trace elements (Fe, Al, Mn, Cu, Zn, Ni, Mo and Sr). Pb, Co, Cd, As and Hg were absent or were beyond the determination capabilities of the apparatus and, hence, established was a conformity of dry extracts to the permissible limits of heavy metals specified in State Pharmacopoeia of Ukraine.

The total content (mg/100 g) of elements in extract **I** was 6255.56, in extract **II** was 6487.03, and the highest contents of both macro- and trace elements were observed in extract **II**. Quantitatively (mg/100 g), K (5040), Ca (630), Na and Mg (250) prevailed in extract **I**; K (4170), Si (1040) and Mg (835) dominated in extract **II**; extract **I** and **II** significantly differed in calcium (630 and 70, respectively), silicon (8 and

1040, respectively), zinc (0.8 and 7.0, respectively) and nickel (0.84 and 0.13, respectively) content.

The descending series of elements were characteristic for the extracts studied and were the following: for extract **I**, K>Ca>Na=Mg>P>Si>Mn>Ni>Fe>Al>Zn>Sr>Cu>Mo; for extract **II**, K>Si>Mg>Na>P>Ca>Zn>Al>Cu>Mn>Fe>Sr>Ni>Mo.

Table 1

The elemental composition of dry extracts from *Galium verum* herb

Element	Element content, mg/100 g	
	Extract I	Extract II
K	5040	4170
Na	250	280
Ca	630	70
Mg	250	835
Si	8	1040
P	71	80
Fe	0.8	0.7
Al	0.8	1.4
Mn	2.1	0.7
Cu	0.42	1.4
Zn	0.8	7.0
Ni	0.84	0.13
Sr	0.8	0.7
Total	**6255.56**	**6487.03**

Note: Mo, Pb and Co<0,03, Cd<0,01, As<0,01 and Hg<0,01.

Also, the plant to extract transfer factors were determined (Table 2).

Table 2

The plant to extract transfer factors for macro and trace elements

Element	Transfer factor	
	Extract I	Extract II
K	0.40	0.36
Na	0.35	0.42
Ca	0.20	0.02
Mg	0.23	0.84
Si	<0.0001	0.39
P	0.11	0.13
Fe	<0.0001	<0.0001
Al	<0.0001	<0.0001
Mn	0.02	0.01
Cu	0.14	0.53
Zn	0.01	0.11
Ni	0.39	0.07
Sr	0.04	0.04

It is known that the liver damage by carbon tetrachloride is associated with trichloromethylperoxy radicals formation and further lipid peroxidation, destruction of polyunsaturated fatty acids and subsequent lowered membrane permeability of all cellular compartments, generalized hepatic damage, followed by inflammation and fibrosis [10].

Numerous *in vivo* studies showed protective effects of macro and trace elements in different animal models of liver injury, associated with lipid peroxidation, inflammation and abnormalities in liver histology.

Zinc treatment to animals with induced liver injury normalized raised levels of lipid peroxidation; resulted in an elevation of important components of with known antioxidant and detoxifying properties, namely reduced glutathione, catalase, glutathione-S-transferase and metallothionein levels, overall improvement in the hepatic histoarchitecture was observed [5].

A biologically active copper-chelating complex, copper-nicotinate, possesses detoxifying action attributed to scavenging of free radicals, exhibiting superoxide dismutase-mimicking activity [6].

A long-term administration of *Salvia miltiorrhiza* ameliorated carbon tetrachloride-induced hepatic fibrosis in rats [7], and recent studies showed that magnesium lithospermate B (MLB), one of the major active components of *Salvia miltiorrhizae,* strongly suppressed H_2O_2-induced reactive oxygen species generation and inhibited type I collagen secretion in hepatic stellate cells. Authors concluded that MLB has potent antifibrotic effect and inhibits fibrogenic responses in hepatic stellate cells [8].

Silicon-enriched diet enhanced the liver antioxidant status, reduced hepatosomatic index and increased superoxide dismutase activity [3].

Comparing to extract **I**, extract **II** is characterized by the higher concentration of copper, magnesium, zinc and silicon, elements that improve liver antioxidant and anti-inflammatory defences. The concentrations of copper, magnesium, zinc and silicon in extract **II** were 3.33-, 3.34- 8.75- and 130-fold higher than those in extract **I.**

Imbalance in trace elements may contribute to oxidative stress and cell death of hepatocytes. Mitochondrial calcium regulates apoptotic processes: calcium overload can lead to the swelling of mitochondria and to the rupture of the outer membrane, in turn releasing proapoptotic molecules in the cytosol [4].

Toxicity induced by nickel sulfate in rat liver was characterized by significant increase in lipid peroxidation and alterations in normal hepatic histoarchitecture. Administration of zinc to nickel treated rats improved enzymes activities and the structure of hepatocytes, demonstrating the ability of zinc to restore the altered hepatic histoarchitecture. The authors concluded that zinc has the potential in alleviating the toxic effects of nickel in rat liver because of its property to induce metallothionein (S-rich protein) as a free radical scavenger, or its indirect action in reducing the levels of oxygen reactive species [9].

Extract **II** is characterized by the lower concentration of calcium and nickel compared to extract **I**. The concentrations of calcium and nickel in extract **II** were 9- and 6.46-fold lower than those in extract **I.**

Conclusions. The higher content of copper, magnesium, zinc and silicon in combination with significantly lower content of calcium and nickel in extract **II** partially explain more pronounced hepatoprotective activity of the extract **II** comparing to extract **I.**

Research into measuring of oxidative stress biomarkers and antioxidant enzymes levels is required in order to give more detailed explanation of the role of macro and trace elements in established hepatoprotective effects of dry extracts from *G. verum* herb.

Rich elemental composition of dry extracts from *Galium verum* L. herb gives theoretical background for further research into other pharmacological activities, namely anti-inflammatory, immunomodulatory, sedative and hypoglycemic.

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