ON THE PHARMACOGNOSTICAL STUDY OF ROSA MAJALIS ROOTS

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ABSTRACT

AIM: The aim of the research was the study of the amino acid content of *Rosa majalis* root as a new perspective source of these substances.

MATERIALS AND METHODS: The roots of *Rosa majalis* which were prepared in the autumn of 2014 were the object of study. The amino acid content of the raw material was determined by chromatography-mass spectrometry (chromatograph Agilent Technologies, model 1100, USA).

RESULTS AND CONCLUSION: As a result of the research, 21 amino acids were identified in the roots of *Rosa majalis*. From the amino acids it was determined that there was a significant amount of leucine, alanine, and glutamic and aspartic acid.

Keywords: *rosa majalis, root, amino acids*

INTRODUCTION

Rosa majalis is a widespread plant the fruits of which are used in scientific and folk medicine for their vitamin and anti-inflammatory activity. The roots, leaves and flowers are used in folk medicine only. We paid attention to the roots which have anti-inflammatory, bactericide and choleretic activity. They help to take out nephroliths and normalize the metabolic processes in the organism. The decoctions and infusions are used to treat diseases of the gastrointestinal tract, liver and kidneys and inflammatory diseases of the urogenital and locomotor systems (3,4). Although the roots have been widely used in folk medicine of different countries for a long time, their chemical content is poorly studied. Analysis of literature sources shows that this raw material contains organic acids, triterpenoids, flavonoids, tannins, catechins and lignans (3,4). No information about amino acids in the root of *Rosa majalis* was found, that is why the studying of the qualitative composition of amino acids in raw material is of importance as they play an essential role in the vital activity of live organisms and are included in the compositions of drugs such as glutargin (arginin glutamate), betargin (contains arginin and betain), aspargit (contains arginin), leucine, tryptophan, glutamic acid, etc. (5).

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AIM

The aim of the research was to study the amino acid content of *Rosa majalis* roots as a new perspective source of these substances.

MATERIALS AND METHODS

The roots of *Rosa majalis,* which were prepared in the botanical garden of the National University of Pharmacy, in the autumn of 2014 were the object of study. The amino acid content of raw material was determined by chromatography-mass spectrometry (chromatograph Agilent Technologies (model 1100,

USA) with running vacuum degasifier G1379A, four-channel pump of low pressure gradient G13111A, automatic injector G1313A, thermostat of column G13116A and diode matrix detector G1316A) (2). The chromatographic column was 4.6m x 0.005m, the sorbent was oxydecylsilyl, granulation - 1.8mkm, "ZORBAX-XDB-C18".

Conditions of chromatography

The gradient mode was on, the working pressure of eluent was 220-275 kPa, the temperature of column thermostat was 50^{0}C, the volume of sample was 2 mcl.

Characteristics of definition

The scale of dimensions was 1.0, time of scanning was 0.5s, the mobile phase velocity was 1.5-2.0 ml/min. The wavelength of detection was 265 nm. The solvents were: A – 0.05M aqua solution of sodium acetate, pH 6.5; B – 0.1M aqua solution of sodium acetate: ACN = 23:22 v/v, pH 6.5; C – aqua and D – acetonitrile (2).

Sample preparation

Sample 1 (free amino acids): 3 ml of mixture of 0.1N aqua solution of hydrochloric acid and 0.2% ß-mercaptoethanol was added to 0.3 g (exact weight) of the raw material which had been powdered and weighed in vial (the volume of the container was 10 ml). The seal of the vial was airtight and it was kept in the ultrasonic bath for 2h at temperature of 50^{0}C.

Sample 2 (free and combined amino acids): 3 ml of mixture of 6 N aqua solution hydrochloric acid and 0.4% ß-mercaptoethanol was added to 0.2 g (exact weight) of powdered and weighed in vial (the vol-

Table 1. Content of amino acids in Rosa majalis root (mg/100g in terms of absolutely dry raw materials)

№	The name of amino acid	Content of amino acid	
		free	bound
Essential amino acids			
1	Valine	5	78
2	Isoleucine	6	70
3	Leucine	5	207
4	Lysine	7	96
5	Methionin	12	4
6	Threonine	7	118
7	Phenylalanine	4	105
The sum of essential amino acids		46	678
Relatively essential amino acids			
8	Tyrosine	-	51
9	Cysteine	15	1
10	Histidine	7	38
11	Alanine	10	148
The sum of conditionally essential amino acids		**32**	**238**
Nonessential amino acids			
12	Arginine	46	327
13	Asparagine	46	-
14	Glutamine	3	-
15	Aspartic acid	34	448
16	Glutamic acid	7	867
17	γ-aminobutyric acid	30	3
18	Glycine	5	216
19	Proline	25	157
20	4-hydroxyproline	-	62
21	Serine	22	206
The sum of nonessential amino acids		**218**	**2286**
Total sum of amino acids		296	3202

ume of the container was 10 ml) raw material. The vial with an airtight seal and kept in the ultrasonic bath for 2h at temperature of 110^{0}C.

The vials with sample 1 and 2 were centrifuged and the raw materials were filtered. The reaction vials with the volume of 2 ml each contained 100 mkl of the 1st sample filtrate and 20 mkl of the 2nd sample filtrate. The vials were put in a vacuum desiccator (temperature 40-45^{0}C, pressure 1.5 mmHg) for the absolute removal of the hydrochloric acid. Then, with the help of an automatic dosimeter, 200 mkl of 0.8 M borate buffer with pH 9.0 and 200 mkl 20M solution of 9-fluorenylmethoxycarbonyl chloride in acetonitrile were added consequently. The vials were kept for 10 min and then 20 mkl 150M solution of amantadine hydrochloride in aqua solution of acetonitrile were added each time.

RESULTS AND DISCUSSION

The results from determining the quantitative composition of free and bound amino acids and their quantitative content are shown in Table 1 and their

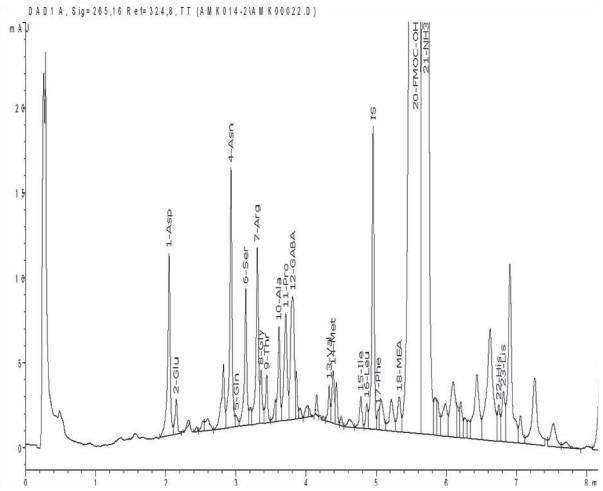

Fig. 1. A chromatogram of free and combined amino acids of Rosa majalis roots

chromatograms are on Figure 1.

There were 21 amino acids identified in the roots of *Rosa majalis*. There were 7 essential, 4 relatively essential and 10 nonessential amino acids. The sum of bound amino acids (3202mg/100g in terms of absolutely dry raw materials) was more than 10 times higher than the sum of free amino acids (296 mg/100 g in terms of absolutely dry raw materials). Among the essential amino acids was a significant amount of leucine, which has immunostimulatory and anabolic action (1). There was a high content of alanine (it-strengthens the immune system developing antibodies and takes part in the metabolism of sugars and organic acids) from the relatively essential amino acids. From the nonessential amino acids, glutamic acid (stimulates transmission of excitation in the synapses of central nervous system) and aspartic acid (fills up deficiency of magnesium and calcium, normalizes electrolytic balance and has antiarrhythmic action) were determined. The total content of amino acids in roots of *Rosa majalis* was 3498mg/100g, which was 3.5%.

CONCLUSION

1. For the first time, the qualitative composition and quantitative content of free and combined amino acids in *Rosa majalis* roots were studied.
2. It was determined that the raw material contained more of the combined amino acids than the free ones.
3. As a result of studying the total content of glutamic acid (874 mg/100 g), aspartic acid (482 mg/100 g), leucine (212 mg/100 g) and alanine (158 mg/100 g) it may be assumed that *Rosa majalis* roots can have an immunostimulatory activity.

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MERCURY SELF-POISONING. CASE REPORT

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ABSTRACT

A clinical case of a self-poisoning with a single ingestion of 40-45 mL of alleged metal mercury with suicidal purpose by a 50-year-old man is described. On the following day he was admitted to the Toxicology Clinic with symptoms of nausea, strong abdominal colic, diarrhea, and feces with macroscopic admixture of mercury drops. At the inspection of the residue of the substance, an unusual black-grey color on its surface was noted. Later, mercurous oxide was proven by chemical analysis. No toxic symptoms of the central nervous system, respiratory system or kidneys were observed. X-rays of the abdomen were performed and tracked dynamically: the first one showed numerous round shadows with metal density along the whole colon, the second - after 5 days - showed reduced number of similar shadows only in the distant colon, and the third X-ray on the 9th day was normal. Mercury was discovered in the blood: 0.250 μmol L^{-1} on the fourth day after the ingestion and 0.120 μmol L^{-1} - on the tenth day. Some therapeutic problems of acute mercury intoxication of present interest are discussed.

Keywords: *mercury metal, inorganic mercury compounds, mercurous oxide, ingestion, gastrointestinal syndrome*

INTRODUCTION

Mercury poisoning has been well known for a long time. Mercury is a heavy silvery-white metal, liquid at room temperature. It is a cell and protoplasmic toxicant that binds the sulfhydryl groups of proteins and leads to protein precipitation, damage of the cell membranes, reduction of RNA in the cells and blockage of a number of important enzyme systems (1-5). It is toxic in all forms: metal mercury, inorganic and organic mercury compounds (2,5-7). The sources of poisoning, toxicokinetics and biological effects vary significantly depending on these forms; therefore, the mercury toxicity is also highly varied (2,3,7-10). The main factors for this variety are chemical form, route of exposure (ingestion, inhalation and dermal), duration of exposure (acute or chronic), and the dose and intensity of the exposure (2,10). Metal mercury is dangerous mainly through inhalation, while its ingestion is considered relatively safe (4,10-12). In toxicological literature the opinion that metal mercury is not absorbed by the gastrointestinal tract with intact membranes and has insignificant absorption – less than 0.1% of the ingested amount – prevails. Some cases of ingestion of significant amounts of metal mercury with suicidal purpose – from 204 g to 3.0 kg (220 mL) without substantial toxic effects – have been described (5,6,9,11,13-15). The authors emphasize on the importance of the normal peristalsis of the bowels and the intact intestinal membranes (10,16). Inorganic mercury compounds are mercu-

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rous salts (monovalent) and mercuric salts (bivalent). They enter the blood circulation only by ingestion. They are lipid insoluble, have various degrees of water solubility and do not pass through the hematoencephalic barrier. At first, the soluble mercuric salts are absorbed moderately (7-15%) and a significant part of Hg^{2+} can stay unabsorbed, attached to the alimentary mucosa or in the intestinal content, waiting for absorption later. Mercurous salts are not soluble at first, have restricted absorption, but they can undergo oxidation to soluble compounds (2,7,10). Inorganic mercury compounds have an irritating-corrosive effect on the mucosa of the gastro-intestinal tract and especially on the intestines. After the absorption, they accumulate mainly in the liver and kidneys. In the early phase, the mercury in the blood is about 1-1.5% of the ingested dose. Over 90% of the inorganic mercury in the blood is bound to the erythrocytes and proteins and less than 1% is unbound. Its volume of distribution is approximately 20 L/kg^{-1}. It is eliminated mainly via the kidneys by glomerular filtration and tubular secretion and also via biliary excretion, with the feces. The third form, organic mercury compounds, is highly liposoluble, pass through the hematoencephalic barrier and cause a severe cerebral toxic syndrome (5,9,17,18). Organic mercury compounds are not a subject of this article, but their impact on human health continues to be an international problem (4,5,9,13,17-20).

We describe a clinical case of a single-time ingestion of a significant amount of initially reported pure metal mercury, about 40-45 ml, with suicidal purpose, by a 50-year-old man who was in good health before that. The diagnosis was formed on the basis of the history, clinical toxic syndrome, the residue of the substance in the vial, the toxicology laboratory results and X-ray images.

MATERIALS AND METHODS

Hospital case 4256/2016. Macroscopic inspection and classical wet chemistry semi-micro qualitative analysis of the residue of the substance found at the bottom of the brought vial in the Laboratory of Analytical Toxicology in Naval Hospital – Varna were conducted. Quantitative toxicological chemical analysis of the patient's blood for mercury was done at the Analytical Laboratory in St. Ivan Rilsky Hospital, Sofia. Native X-ray of the abdomen were performed on 31.08.2016, 04.09.2016 and 07.09.2016.

RESULTS

Anamnesis

R.Y.M., a man, 50 years old, from the village of G., Varna District, was admitted to the Toxicology Clinic on 31.08.2016 with a history of strong abdominal cramps, nausea and diarrhea with admixture of blood which started about 24 hours after a single ingestion of alleged metal mercury, with suicidal purpose. The patient said that the mercury had been taken many years ago from a nowadays non-existing department of the Polyvinylchloride Production Plant in the town of Devnya and was kept in a well-sealed 50 mL glass vial. Before the intoxication, the patient was in good health. Physical examination: conscious, oriented, tense, with dysthymia. No ataxia, convulsions or negative neurological symptoms, normal muscle tonus. There were normal color and turgor of the skin and oral mucosa. The respiratory rate was 24 per minute. The examination also showed: vesicular breathing, no wheezing; bradycardia 45/min, rhythmic; later 65/min. The arterial blood pressure was normal. The abdomen was soft with painful palpation in the lower abdominal half, along the colon. There were no symptoms of peritoneal irritation; no organomegaly and non-painful renal succussion.

Diagnostics

Macroscopic inspection of the original vial (Fig. 1) has shown approximately 1 mL of liquid, visibly non-clean mercury (Fig. 2). Several milligrams of fine black powder were found as a residue in the vial

Fig. 1. The original vial

Fig. 2. Liquid mercury leftover

Fig. 3. Black powder found together with mercury in the original bottle

(Fig. 3). Classical wet chemistry semi-micro qualitative analysis has proven mercury (I) oxide as its principal component. Toxicological chemical analysis of blood for mercury was performed: 03.09.2016 (4th day after ingestion) – 0.2500 μmol L^{-1}; 08.09.2016 (10th day) – 0.1200 μmol L^{-1}. The results were received after the discharge of the patient from the hospital.

Routine laboratory tests showed results within normal ranges. The ECG data were: on admittance – sinus bradycardia 44/min, indifferent cardiac position. Control ECG-s showed sinus rhythm. The X-ray of the lungs and heart was normal. The X-ray of the abdomen showed the following: on 31.08.2016 –

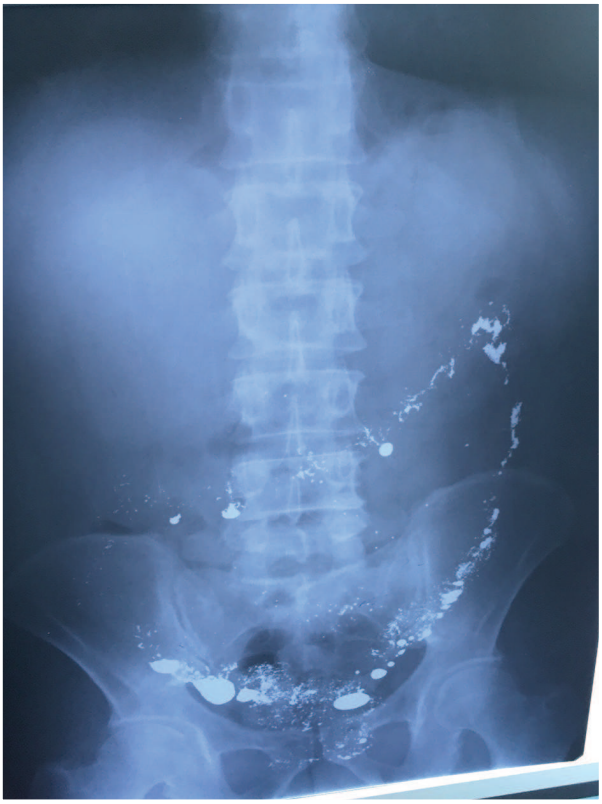

Fig. 4. X-ray on 31.08.2016: numerous X-positive round shadows with metal density along the colon – "mercury necklace"

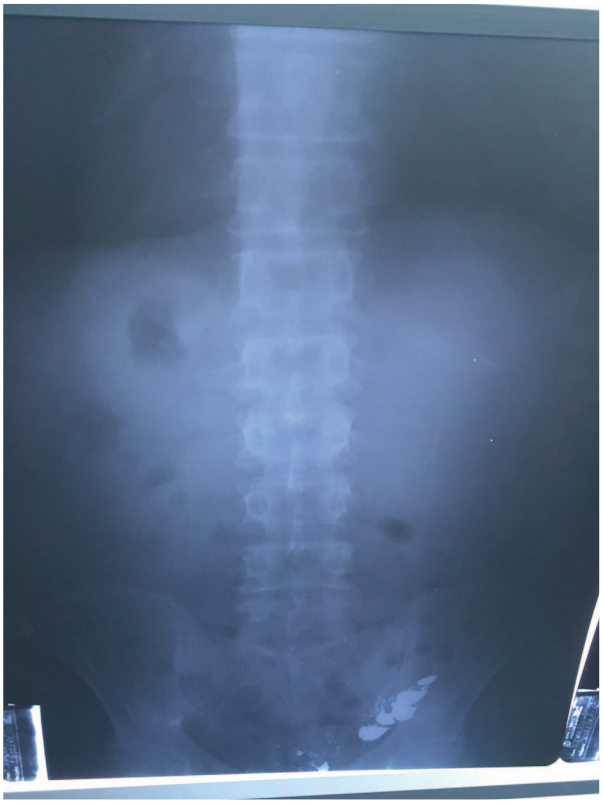

Fig. 5. X-ray on 04.09.2016: reduced number of positive shadows along the descendant colon

numerous X-positive round shadows with metal density along the colon (Fig. 4); on 04.09.2016 – reduced number of similar shadows along the descendant colon (Fig. 5); and on 07.09.2017 – normal image of the abdomen, no pathologic shadows.

The ultrasound diagnostic of the abdomen showed a blurred boundary between renal cortex and pyelonephritis of both kidneys. The ultrasound image of the other abdominal organs was normal. Fibrogastroscopy and fibrocolonoscopy were planned, but refused by the patient. A consultation with a surgeon determined that there were no symptoms of an acute abdominal surgical problem. A consultation with a psychiatrist lead to the diagnosis - disorder of adaptation. There was a protracted depressive reaction and a suicide attempt. The patient was directed to the Psychiatric Department of the St. Marina University Hospital in Varna, but declined to be admitted there.

Treatment

We have performed stomach lavage, bowel irrigation and used osmotic laxative repeatedly, intravenous infusion of electrolyte and glucous solutions, H2-blocker – Quamatel (famotidin) IV, inhibitor of the proton pump – Ulcoprol (omeprazol), spasmolytics, analgesics, antibiotic – Ceftriaxon IV, and included a diet as well. Antidote treatment was delayed due to deficit of Dimercaprol.

Clinical course: during the first week the patient suffered from constant abdominal pain, mainly cramps along the colon, with temporary effect of spasmolytics. After the 6th day, the intensity of the abdominal symptoms was reduced. Until the 5-6th day, mercury particles could be macroscopically seen in the feces. On the 9th day the patient was better, without cramps or diarrhea and was discharged on his own will, with prescription and dietetic advice. Three days later, he phoned about renewed appearance of abdominal cramps and diarrhea, possibly with blood admixture, without any laxatives. He was admitted to the Gastroenterology Department of another hospital for diagnostic gastroscopy and colonoscopy, but once again refused these procedures and left on his own will. He did not return to the Toxicology Department of the Naval Hospital for re-admittance either and missed all the dates for control examination. Several months later, on the telephone, his relatives declared that he had refused any medical help but was "well".

DISCUSSION

The described clinical case created several diagnostic and therapeutic problems from the beginning. Although we had a clear history of ingestion of a significant amount of pure metal mercury, confirmed by the X-ray of the abdomen, the clinical presentation with severe gastroenterocolitis and later, the high mercury blood levels found, were in contradiction with the expected lack of absorption, none or minimal local effects and lack of serious toxicity of the ingested metal mercury. It resembled inorganic mercury oral intoxication. When the vial was brought, the macroscopic inspection showed visible residue – fine grayish-black powder. Classical wet chemistry semi-micro qualitative analysis proved mercury (I) oxide as its principal component. According to the literature, the metal mercury may oxidize to mercury oxide in nature when there is moist air, forming a film of Hg_2O, but it is a very slow process (20). Later, the presence of Hg_2O as impurity in liquid mercury has found its reasonable explanation, as the industrial origin (electrochemical application) of this specific sample has been confirmed. The mercury poisoning in this case was with mixed mercury forms: inorganic compound and metal mercury, with prevailing inorganic mercury toxic syndrome. The mercury blood level was higher than expected. Additional route of exposure – inhalation of metal mercury vapours before the oral ingestion was discussed, but the patient denied such possibility and no central nervous or respiratory toxic symptoms were observed. The following differential diagnoses were discussed: (i) Ingestion of metal mercury, contaminated by inorganic mercury compound/s; (ii) Ingestion of pure metal mercury by a patient with pre-existing disease of the gastrointestinal tract with loss of intact intestinal mucosa; (iii) Combination of metal mercury ingestion and inhalation of mercury vapours and (iv) Combined intoxication by unknown toxin with gastrointestinal disturbing effects and ingestion of metal mercury. Treatment was mainly depuration and symptomatic at first. The existing deficit of antidotes for heavy metal intoxications has delayed antidote treatment. Additional obstacle was created by the negative attitude of the patient to some

diagnostic and therapeutic procedures. The possible delayed toxic gastrointestinal and kidney symptoms in this case could not be followed and treated properly because of his refusal of control examinations.

CONCLUSION

Although mercury intoxication, especially with suicidal purpose, is rare in Bulgaria, this case showed that some amounts of industrial mercury can be kept for years and used with significant toxic effects. The mercury poisoning in this case was mixed – with two different mercury forms: inorganic compound (Hg_2O) and metal mercury, with leading clinical presentation of Hg_2O toxic syndrome. Chemical examination played a very important role for the right diagnosis because of the high toxicity of the inorganic compound. The described case was a diagnostic challenge at the admittance of the patient and confirmed the necessity of strict verification of the form of mercury in mercury poisonings, based on the history, clinical toxic syndromes and toxicological chemical analysis.

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