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CONTENTS

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EFFECT OF MAGNEGITA ON SELECTED BLOOD PARAMETERS

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ABSTRACT
Magnetic resonance imaging (MRI) is a noninvasive diagnostic test widely used in clinical practice. Signal resolution and sensitivity can be further enhanced by adding contrast agents in order to make abnormalities or disease processes more visible. Gadopentetate dimeglumine was the first approved gadolinium-based contrast agent (GBCA) and showed good diagnostic value and high levels of safety and tolerance. However, if the free gadolinium (Gd) ion is released *in vivo,* cardiovascular and neurologic toxicity may occur. The plasma half-life of the Gd chelates is dependent upon the volume of distribution and the glomerular filtration rate of the agent, and it is higher in patients with impaired renal function. This is why both FDA and EMEA recommended caution when using GBCA-enhanced MRI in patients with moderate renal impairment and banned their use in those with severe renal impairment. The aim of this study was to assess the effect of Magnegita on certain laboratory parameters in patients with normal renal function undergoing cerebral and abdominal MRI scans. Twenty-one patients were included from whom we collected three blood samples (prior to MRI scan, at 30 minutes and 24 hours after). We have assessed 27 parameters by the means of hematology, biochemistry and ionogram tests. Our results showed minor and transient changes in some of the parameters, but most of them remained unchanged throughout the study. Further studies are needed to determine the effect of gadopentetate dimeglumine on the same laboratory parameters in patients with impaired renal function.
Key words: Magnegita, gadopentetate dimeglumine, laboratory parameters

INTRODUCTION

Magnetic resonance imaging (MRI) is a noninvasive diagnostic test widely used in clinical practice as it allows for the simultaneous examination of the anatomy and physiological parameters of tissues and organs by measuring the radio-frequency signals emitted by water protons in living tissues, without using harmful radiation [1,2]. In order to make abnormalities or disease processes more visible, the signal resolution and sensitivity need to be enhanced and this is achieved by adding contrast agents (CAs); therefore approximately 35% of MRI scans are currently performed with the use of CAs [3, 4]. Gadolinium (Gd), a rare-earth metal of the lanthanide series, is the most widely used metal for the production of CAs, being administered in the form of chelate complexes in order to prevent the release of toxic metal ion [5] The free gadolinium ion has cardiovascular and neurologic toxicity, being deposited in liver, bone and lymph nodes [6].

Gadopentetate dimeglumine (Gd-DTPA) was the first approved gadolinium-based contrast agent (GBCA) [7,8]. Early studies [9-11] evaluating the safety and efficacy of Gd-DTPA have concluded it has higher diagnostic value compared to the non contrast-enhanced MRI and exhibits high levels of safety and tolerance. In a multicenter double-blind randomized clinical trial [9], Gd-DTPA improved diagnostic ability in 65% of patients and led to changes in presumptive diagnosis, along with visualization of more lesions in some of those patients. Adverse reactions in some patients were reported, such as asymptomatic rise in serum iron and bilirubin levels, hyper- or hypotension, weakness, conjunctivitis, local burning at injection site, headache, nausea and vomiting, all of which were minor and transient. These adverse reactions were comparable to those related to administration of iodinated non-ionic roentgen contrast media [12].

However, the plasma half-life of the Gd chelates is dependent upon the volume of distribution and the glomerular filtration rate of the agent, and it is higher in patients with impaired renal function [13]. Contrast-induced nephropathy, defined as increase in creatinine concentration [14] or decrease in glomerular filtration rate [15] has been associated with GBCAs, particularly at high doses and in patients with renal impairment. Moreover, nephrogenic systemic fibrosis (NSF), a life-threatening fibroproliferative

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disease seen in people with severe renal impairment, was first observed in 1997 [16] and it has been associated with the use of GBCAs. NSF is thought to be caused by the deposition of free gadolinium in tissues of patients with severe renal dysfunction and the subsequent inability of kidneys to eliminate it because of poor water solubility of the gadolinium [17]. Consequently, in 2007 the USA Food and Drug Administration requested manufacturers to add a warning box to the labels of all GBCAs, stating the increased risk of NSF in patients with acute or chronic severe renal insufficiency [18], while the European Medicines Agency (EMEA) has released in 2009 a set of recommendations [19], classifying the active substances in the GBCAs into three categories of risk: high-, medium-, and low-risk. EMEA's Committee for Medicinal Products for Human Use has recommended contraindications in patients with severe kidney problems, in those scheduled for or who had recently received a liver transplant and in newborn babies up to four weeks of age for high-risk gadolinium-containing contrast agents, including Magnegita. Additionally, patients with unknown kidney problems should always be screened for kidney problems using laboratory tests before the use of a GBCA [19]. Finally, a wide variety of changes in physiology may occur following the administration of gadolinium salts and chelates [20, 21]. However, most of the studies have focused on the effects of GBCAs on kidneys, while just a few have specifically addressed the physiological changes triggered by these agents.

The aim of this study was to assess the effect of Magnegita on certain laboratory parameters in patients with normal renal function undergoing cerebral and abdominal MRI scans.

MATERIALS AND METHODS

The study group was composed of 21 patients who underwent cerebral and abdominal MRI scans in the Radiologica Plus Medical Imagistics Centre Timisoara in the period January - March 2015. No patient has had kidney or liver impairment.

Inclusion criteria: patients aged 20-60, both genders; not pregnant or breastfeeding, in case of women; had not had an organ transplant; had not received any medications within 48 hours of participation; had not received any contrast agent within 7 days; willing to sign Informed Consent Form; willing to provide three blood samples.

Exclusion criteria: patients with impaired renal function; patients who have had a contrast-enhanced MRI or CT seven days prior to inclusion in the study; under chemo- or radiation therapy; highly elevated or decreased laboratory parameters.

Most of the patients (18; 7 women, 11 men) were under 40 years of age, while the other three (2 women, 1 man) were aged over 40 (Figure 1). The number of men included was slightly higher (Figure 2).

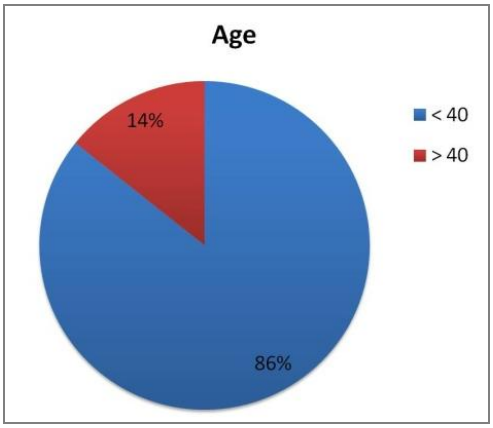

Fig. 1. Distribution of patients by age group

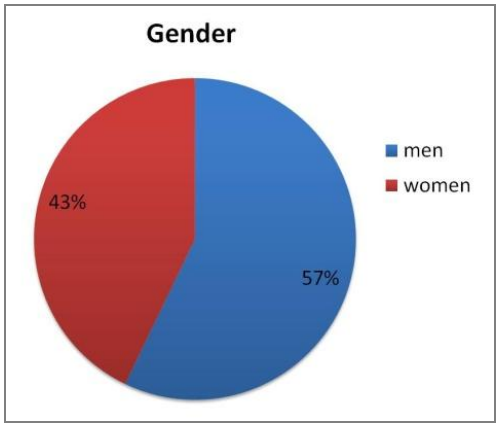

Fig. 2. Distribution of patients by gender

All the patients in this study received 0.1 ml/kg body weight gadopentetate dimeglumine (Magnegita® 500 micromol/ml, Biokanol Pharma GmbH) by intravenous injection once and the exposure to the contrast agent was not repeated in any of the patients. As the use of GBCAs has been linked to the risk of developing nephrogenic systemic fibrosis, all the patients are screened for renal dysfunction prior to be administered Magnegita. None of the patients included in our study suffered from moderate (GFR 30–59 ml/min/1.73 m^2) or severe (GFR < 30 ml/min/1.73m^2) renal impairment.

After the patients have signed the Informed Consent Form to be included in this study, two blood samples of 3 ml (for complete blood count) and 6 ml (for biochemistry tests), respectively, were collected from each patient for three times, namely prior to MRI, then 30 minutes and 24 hours after the MRI scan. We have analysed the same 27 parameters (see Table I) by the mean of complete blood count, biochemistry and ionogram tests. We have used reagents from Diagon for hematology tests, DiaSys

Diagnostic Systems for biochemistry tests and Medica for ionogram tests. All reagents were used according to the manufacturer's instructions.

Table I. Methods and parameters studied

Type of laboratory test	Method/Equipment	Parameter
Complete blood count	Laser flow cytometry / SYSMEX SF-3000	white blood cell count (WBC)
		neutrophils (absolute count; percentage)
		lymphocytes (absolute count; percentage)
		monocytes (absolute count; percentage)
		eosinophils (absolute count; percentage)
		basophils (absolute count; percentage)
		red blood cell count (RBC)
		hemoglobin (HGB)
		hematocrit (HCT)
		mean corpuscular volume (MCV)
		mean corpuscular hemoglobin (MCH)
		mean corpuscular hemoglobin concentration (MCHC)
		red cell distribution width (RDW)
		platelets (PLT)
		mean platelet volume (MPV)
		platelet distribution width (PDW)
Biochemistry	Spectrophotometry / HITACHI 917	serum urea
		serum creatinine
		glycemia
		alanine aminotransferases (ALT)
		aspartate aminotransferases (AST)
		total serum proteins
Ionogram	Ion selective electrode technology / EasyLyte Plus	serum calcium
		serum magnesium
		serum sodium
		serum potassium
		serum chloride

RESULTS

At baseline, the WBC level was normal in 20 patients and lower-than-normal in one; no changes were seen in the WBC level of the latter patient following the administration of the contrast agent at the subsequent blood tests. Thirty minutes after the injection of Magnegita, the WBC level slightly increased in one patient and then returned to normal after 24 hours. Additionally, a slightly decreased level was recorded in one patient at 24 hours, although this patient has had normal values both at baseline and 30 minutes post-injection. As mentioned above, the changes were minor, with no clinical significance.

The white blood cell differential has yielded various results. The absolute count of neutrophils, lymphocytes and monocytes has remained unchanged throughout the three measurements. However, the neutrophil percentage was above the normal range limit in three patients at baseline and it remained high after the contrast agent was injected; the only difference noted was in one patient with lower values at 30 minutes and 24 hours. The lymphocyte percentage was in the normal range in 16 patients at baseline (lower values - 3 patients; higher values -2 patients); 30 minutes post-injection, the lymphocyte percentage has reached the normal range in one of the patients who had subnormal values prior to injection of Magnegita, but dropped again at 24 hours, when we also found one more patient with increased values. Distribution of patients with lower, normal or higher neutrophil and lymphocyte percentages across the three measurements is shown in Figure 3.

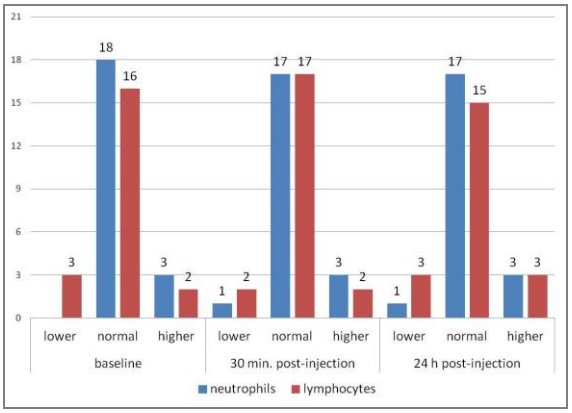

Fig. 3. Distribution of patients according to neutrophil and lymphocyte percentages

The absolute count of eosinophils revealed 11 patients had normal values at baseline, but only five after 30 minutes and 24 hours, respectively, while the other patients (10 at baseline, 16 at the other two measurements) had slightly decreased levels. Nevertheless, the eosinophil percentages exhibited values in the normal range for 17 patients (higher - 4 patients) at the baseline, for 19 patients (higher - 2 patients) 30 minutes after the administration of the GBCA, and for 18 patients (higher - 3 patients) 24 hours post-injection (Figure 4).

Both the absolute count and the percentage of basophils were in the normal range for 20 patients (higher - 1 patient) at the first and second measurements, while at the third measurement, values of 19 patients (higher - 2 patients) remained normal. The monocyte percentage has shown no variations, with one of the patients having decreased values throughout the study.

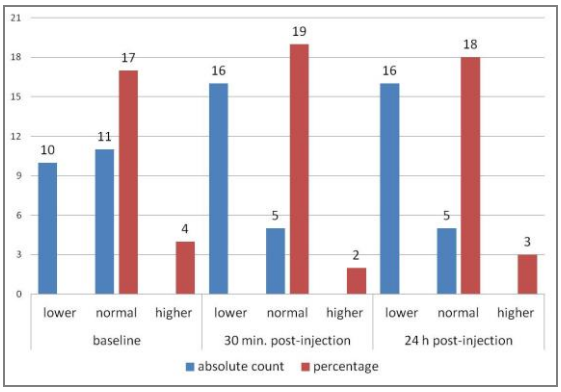

Fig. 4. Distribution of patients according to absolute count and percentage of eosinophils

Red blood cells count showed three patients had lower levels at baseline and remained unchanged at the other two measurements, while the other 18 patients had normal levels. After the injection of Magnegita, RBC dropped in another three patients at the 30 minutes post-injection measurement, but returned to normal after 24 hours. We have also found one patient with higher levels at the 24 h blood test (see Fig. 5). Lower levels of hemoglobin and hematocrit were also seen in four and five patients, respectively, at all three measurements. In three of the patients who had normal levels at baseline, HGB has decreased 30 minutes post-injection, but returned to normal after 24 hours. Similarly, the HCT level has decreased at the second measurement in two patients, but it was normal after 24 hours. The mean platelet volume was higher in three patients at baseline, but it decreased in one of them to a value within the normal range at the subsequent measurements. The platelet distribution width has slightly decreased in one patient 24 h after the injection of the contrast agent. No changes were recorded in mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, and platelets.

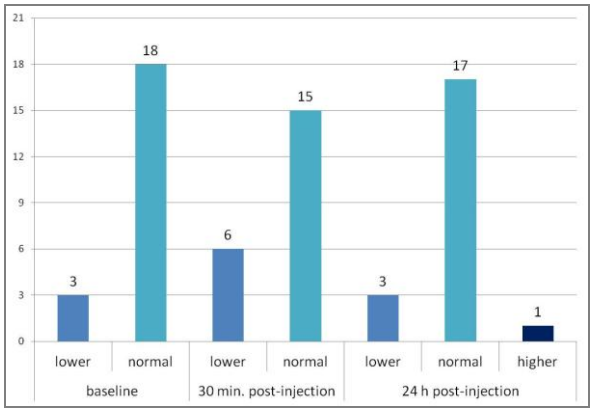

Fig. 5. Distribution of patients according to RBC levels

The biochemistry tests showed no changes in serum urea, serum creatinine, glycemia, and aspartate aminotransferases. Two patients had higher-than-normal levels of alanine aminotransferases at baseline, but only one at the following measurements. The total serum proteins were also higher at baseline in three patients, and then decreased in two of them at the subsequent tests.

The serum calcium level was higher in one patient throughout the study. At the second measurement, it decreased in another patient, while 24 hours later two patients had lower levels (Figure 6). The serum chloride was found to be higher than normal in 12 patients at baseline, and then in 13 and 14 patients at 30 minutes and 24 hours, respectively, after the injection of Magnegita (Figure 7). Serum magnesium, sodium and potassium remained unchanged during the study.

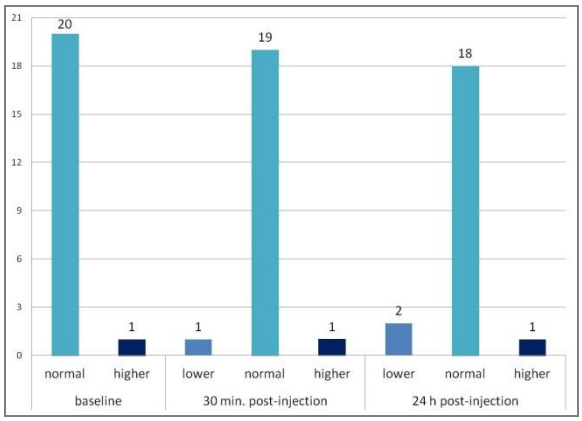

Fig. 6. Distribution of patients according to serum calcium levels

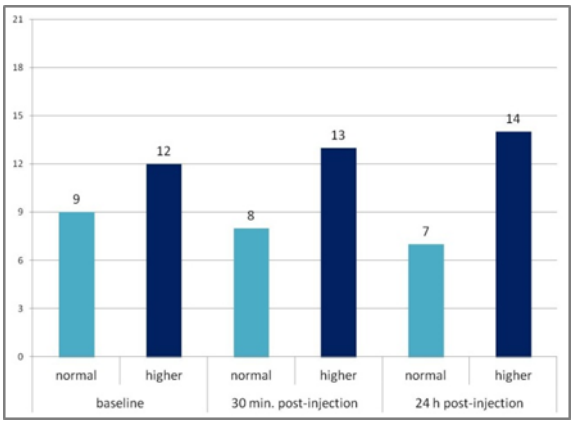

Fig. 7. Distribution of patients according to serum chloride levels

DISCUSSION

Gadolinium chelates are routinely used nowadays in magnetic resonance imaging, mostly for CNS pathology, followed by contrast-enhanced MR angiography, liver imaging, and breast evaluation for malignancy [22]. After intravenous injection, they rapidly distribute in the blood and the extracellular space [13, 23] and are normally eliminated unmetabolized via kidneys through glomerular filtration [13, 24], with half-lives of approximately 1.5 hours in people with normal renal function [5, 13, 23]. Unfortunately, toxic Gd(III) ions may be released *in vivo* by transmetallation with other metal ions, thus the kinetic stability of the gadolinium chelates is very important [3]. The ionic linear Gd chelates (such as gadopentetate dimeglumine) were shown to be more stable than the nonionic linear ones under normal and particularly under altered physiological conditions [25]. Although early studies have found gadopentetate dimeglumine is safe and well tolerated [9-11] in people with normal renal function, it has been later proved that GBCAs may trigger nephrogenic systemic fibrosis in patients with kidney disorders [6, 26, 27]. Therefore, for safety reasons, the

benefits and risks of GBCA-enhanced MRI should be carefully balanced in patients with impaired renal function and also in patients with liver impairment.

A double-blind, randomized, crossover, placebo-controlled study designed to evaluate the safety and tolerance of gadopentetate dimeglumine 0.1 mmol/kg when administered to normal subjects at a four times higher bolus injection rate than that recommended by the producer found no clinically significant changes in laboratory parameters, even a few values outside the specified normal ranges were recorded [28].

Nevertheless, other data in the literature show gadolinium may interfere with various laboratory parameters, although changes are minor and transient. In a retrospective study, data regarding serum calcium, magnesium and creatinine levels were reviewed before and within 1 day after gadodiamide or gadopentetate dimeglumine administration [29]. A transient decrease in serum creatinine was seen in patients with renal impairment who received gadopentetate dimeglumine, but no changes were recorded in serum calcium and magnesium. Conversely, 36.7% of the patients with high-dose gadodiamide injection and renal insufficiency had spurious critical hypocalcemia and minor increases in serum magnesium. Moreover, other *in vivo* and *in vitro* studies have documented the interference with colorimetric assays of gadodiamide and gadoversetamide, resulting in spurious hypocalcemia, but gadopentetate dimeglumine has not been shown to cause this interference [20, 30].

The study by Proctor *et al*. [20] on the effects of gadodiamide, gadoversetamide, gadopentetate dimeglumine, and gadoteridol has shown that GBCAs can produce positive or negative analytic interference with multiple serum assays, including angiotensin-converting enzyme, calcium, iron, magnesium, total iron binding capacity, and zinc, with the first two producing the most interference. Gadopentetate dimeglumine showed interference with iron, while a manual colorimetric zinc assay showed interference with all the GBCAs studied. However, the other parameters measured (41 in total, including some of the parameters we have studied) showed no significant interference with any of the four GBCAs.

Our study results are mostly consistent with these reports. Furthermore, we have found minor and transient or no changes in the other parameters evaluated in this study.

CONCLUSION

Although the risk of developing nephrogenic systemic fibrosis or contrast-induced nephropathy is currently a concern in people with impaired renal function, the use of GBCAs in patients with normal renal function remains safe. Moreover, gadopentetate dimeglumine is among the safest GBCAs and it triggered no clinically significant changes in the laboratory parameters of the patients included in this study. Further studies are needed to determine the effect of gadopentetate dimeglumine on the same laboratory parameters in patients with impaired renal or hepatic function.

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EFECTUL MAGNEGITA ASUPRA UNOR PARAMETRI SANGUINI

REZUMAT

Imagistica prin rezonanță magnetică (IRM) este o metodă non-invazivă de diagnostic larg utilizată în pracica clinică. Rezoluția și sensibilitatea semnalelor poate fi sporită prin adăugarea de agenți de contrast, cu scopul de a crește vizibilitatea anomaliilor sau a proceselor patologice. Gadopentetatul de dimeglumină a fost primul agent de contrast pe bază de gadoliniu (GBCA) aprobat și a dovedit o valoare diagnostic bună, precum și un nivel ridicat de siguranță și tolerabilitate. Totuși, dacă ionul liber de gadoliniu (Gd) este eliberat *in vivo*, poate apărea toxicitatea cardio-vasculară și neurologică. Timpul de înjumătățire în plasmă a chelaților de Gd depinde de volumul de distribuție și de rata de filtrare glomerulară a fiecărui agent de contrast și este mai mare la pacienții cu suferință renală. De aceea, atât FDA, cât și EMEA au recomandat prudență în utilizarea IRM cu GBCA la pacienții cu insuficiență renală moderată și au interzis utilizarea lor la cei cu insuficiență renală severă. Scopul acestui studiu a fost de a evalua efectul Magnegita asupra anumitor parametri de laborator la pacienți cu funcție renală normală care au fost evaluați prin IRM cerebral sau abdominal. În studiu au fost incluși 21 de pacienți, de la care s-au recoltat trei probe de sânge (înainte de IRM, la 30 de minute și la 24 de ore după). Am evaluat 27 de parametri prin intermediul testelor de hematologie, biochimie și ionogramă. Rezultatea noastre au arătat că unii parametri au suferite modificări minore și tranzitorii, majoritatea rămânând neschimbați. Sunt necesare alte studii pentru a determina efectul pe care gadopentetatul de dimeglumină îl are asupra parametrilor de laborator la pacienții cu disfuncție renală.

Cuvinte cheie: Magnegita, gadopentetat de dimeglumină, parametri de laborator

ANALYSIS OF STEROLIC COMPOUNDS FROM VEGETAL EXTRACTS OF *RIBES NIGRUM*, *ROSA CANINA*, *BETULA PUBESCENS*, *CARPINUS BETULUS*, *VIBURNUM LANTANA* AND PROPOLIS TINCTURE

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ABSTRACT

Gemmotherapic vegetal extracts in DH1 dilution of *Ribes nigrum*, *Rosa canina*, *Betula pubescens*, *Carpinus betulus*, *Viburnum lantana*, 30% propolis tincture were analyzed through high performance chromatography coupled with mass spectrometry (LC/MS) to analyze the sterols, specifically: betasitosterol, stigmasterol, campesterol, ergosterol, brassicasterol.

Gemmotherapic vegetal extracts in the DH1 dilution of *Rosa canina* (X1), *Betula pubescens* (X2), *Ribes nigrum* (X3), *Viburnum lantana* (X5), *Carpinus betulus* (X6) and propolis tincture 30% (X4) are frequently used in integrative medicine for their anti-inflammatory properties. Due to the fact that the anti-inflammatory effect of the vegetal extracts may also be induced by the sterols, we proposed to analyze these extracts (obtained from Plantextract, Cluj), through high performance chromatography coupled with mass spectrometry (LC/MS)[1,2,3]. The extracts analyzed were betasitosterol, stigmasterol, campesterol, ergosterol, brassicasterol [1,3]. The concentrations of sterols found through the method above mentioned (expressed in μg/mL) were significant only for betasitosterol in: X1 = 15.859, X2 = 17.251, X3 = 21.598, X4 = 1.516; X5 = 2.033, X6 - unidentifiable.

Keywords: Gemmotherapic vegetal extracts, sterols and propolis, LC/MS/MS analysis

INTRODUCTION

Gemmotherapic vegetal extracts in the DH1 dilution of *Rosa canina* (X1), *Betula pubescens* (X2), *Ribes nigrum* (X3), *Viburnum lantana* (X5), *Carpinus betulus* (X6) and propolis tincture 30% (X4) are frequently used in integrative medicine for their anti-inflammatory and antiallergic properties. Due to the fact that we didn't find research regarding their chemical composition in specialty literature, in this study we analyzed the sterol compounds using high performance chromatography coupled with mass spectrometry (LC/MS), as they are known for anti-inflammatory effect.

MATERIALS AND METHODS

Devices: HPLC coupled with HP1100 Series mass spectrometer with binary pump; HP1100 Series Autosampler, HP1100 Series Thermostat, Agilent Ion Trap 1100 SL Mass spectrometer; Working conditions HPLC: Zorbax SB-C18 analytic column 100 mm x 3.0 mmi, d = 0.5 microns.

Mobile Phase: methanol-acetonitrile mixture, 10:90 (v/v), isocratic elution mode; debit: 1mL/min, temperature: 45C, detection MS/MS, mode MRM; injection volume =5 μL.

Working conditions of MS: ion source: APCI (atmospheric pressure chemical ionization); ionization mode: positive; nebulizer: nitrogen; pressure 60 psi; vaporizer 400C, drying gas: nitrogen; debit 7L/min; temperature = 325C; capillary potential: -4000V.

Analysis mode: monitoring of the sterol specific ions.

Standards: beta-sitosterol, stigmasterol, campesterol and ergosterol

Monitored ions detected through MS methods are presented in Table I. Due to the fact that through ionization all the sterols lose on molecule of water, ions detected by the MS are always in the following form $[M-H_2O+H]^+$.

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Table I. Monitored sterol specific ions in ascending order according to retention time (r.t.)

Sterol	r.t. (min)	M	M-H_2O	M-H_2O+H^+
Ergosterol	2.6	396	378	379
Brassicasterol	3.3	398	380	381
Stigmasterol	4.0	412	394	395
Campesterol	4.0	400	382	383
Sitosterol	4.6	414	396	397

MS detection revealed parent-ions of the analyzed sterols (parent-ions = ions which are formed after ionization before their eventual fragmentation) in each sterol expected ions were revealed (Table I).
Mass spectrum (full-scan type) of the four sterols are presented in Figure 1 (a-e).

This method of analysis (also named MS/MS) is extremely specific as it only registers the intensity of the main ion. An isomer or isobar compound – with the same molecular mass – may give a false positive signal. The analysis on the basis of the fragments from the MS spectrum, which are specific to each structure in turn and which are not the same from one isomer to another, will detect only the compound of interest without interference from others. As well as this, the ion intensity from the MS is proportional with the concentration of the substance in the compound analyzed, so the method may be applied for quantification in place of qualitative analysis.
MS/MS spectrums of the analyzed compounds are presented in Figure 2 (a-e).

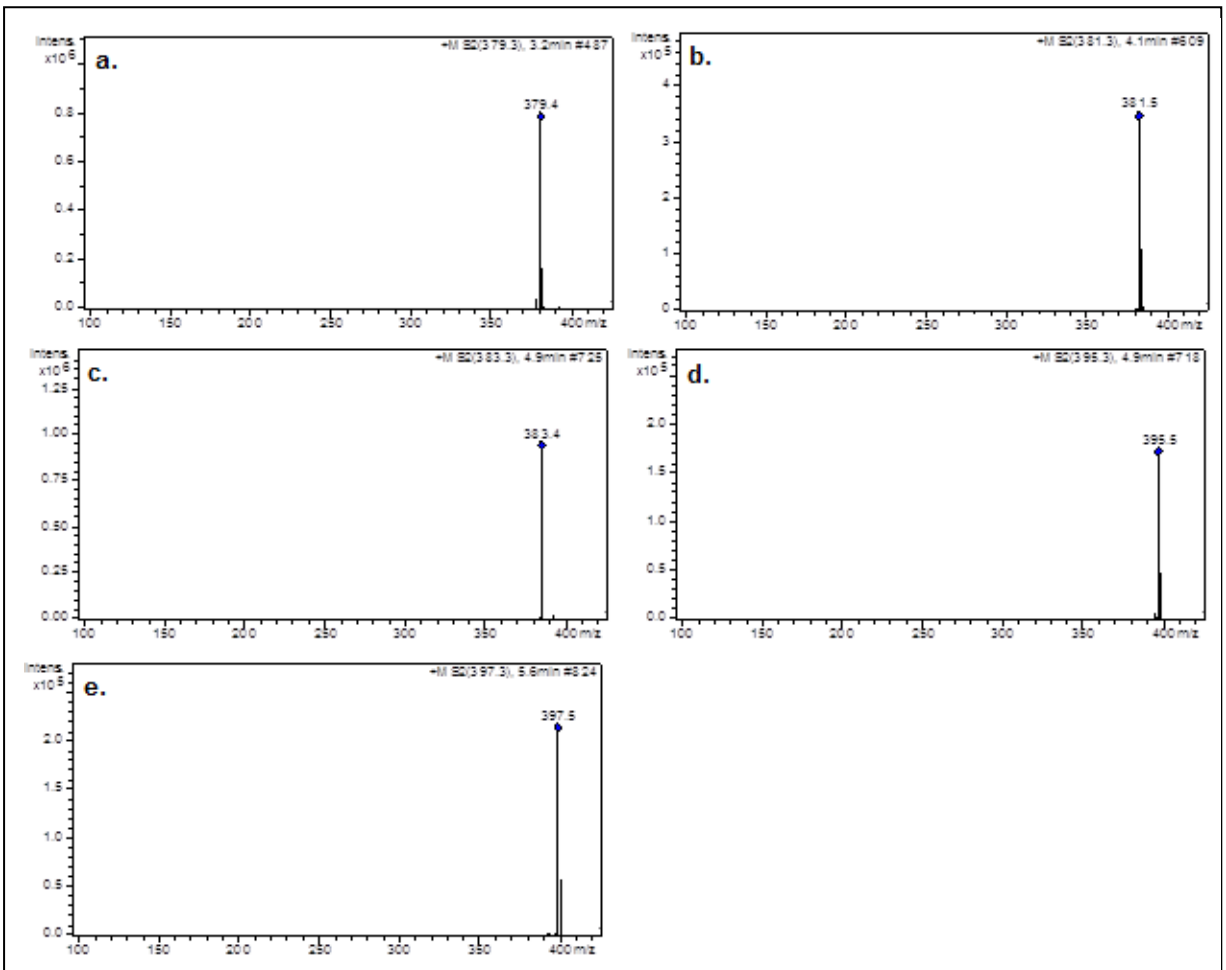

Fig. 1. Full -scan spectrums of the analyzed sterols: a. ergosterol, b.brassicasterol, c.campesterol, d. stigmasterol, e. β-sitosterol

MS/MS spectrums of the analyzed compounds are presented in Figure 2 (a-e).

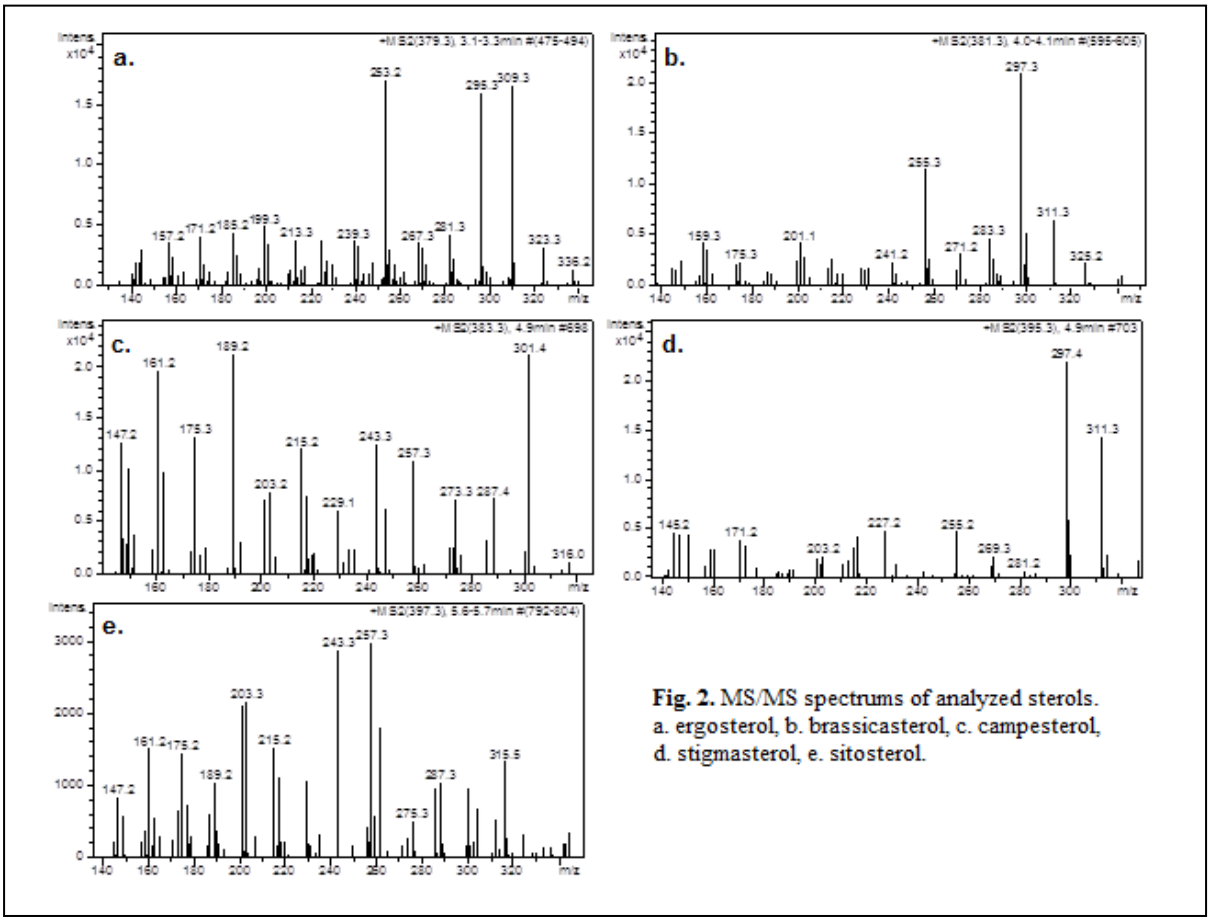

Fig. 2. MS/MS spectrums of analyzed sterols. a. ergosterol, b. brassicasterol, c. campesterol, d. stigmasterol, e. sitosterol.

In order to quantify the analyzed sterols, we built a chromatogram for each of the analyzed sterols, according to the intensity of the principle ions from the MS. The ions used for quantification are numbered in Table II.

Table II. Ions from the MS spectrum of analysed sterols

No.	Compound name	Ions specific for identification
Ion [M-H_2O+H^+] > Ions from spectrum		
1	Ergosterol	379> 253.3,295.3,309.3
2	Brassicasterol	381>201.3,203.3,215.2,217.3,241.2,255.3,257.4,271.1,297.3,299.3
3	Campesterol	383>147.3,149.3,161.3,175.3,189.3,203.3,215.3,229.3,243.3,257.3
2	Stigmasterol	395>163.3,173.2,187.3,199.3,227.2,241.3,255.3,269.2,283.4,297.3
4	Sitosterol	397>160.9,174.9,188.9,202.9,214.9,243.0,257.0,287.1,315.2

For the construction of calibration curves, standard sterols were dissolved in acetone (concentration 1 mg/mL), from which successive dilution in acetonitrile were made in order to obtain the calibration solution. The concentration of calibration solution of each analyzed sterol, concentrations found and the accuracy of the determination are presented in Tables III-VII.

Table III. Concentration of calibration solutions of ergosterol and the accuracy of the determination

Concentration level	Ergosterol		
	Standard concentration (ng/ml)	Concentration found (ng/ml)	Accuracy (%)
1	69.00	70.29	101.87
2	138.00	132.21	95.80
3	276.00	293.67	106.40
4	552.00	514.05	93.12
5	1104.00	1084.81	98.26
6	2208.00	2229.77	100.99
7	3312.00	3534.03	106.70

Table IV. Concentration of calibration solutions of brassicasterol and the accuracy of their determination

Concentration level	Brassicasterol		
	Standard concentration (ng/ml)	Concentration found (ng/ml)	Accuracy %
1	62.00	60.71	97.92
2	123.00	132.74	107.92
3	246.00	258.38	105.03
4	492.00	489.86	99.56
5	984.00	913.66	92.85
6	1968.00	1971.57	100.18
7	2952.00	2931.16	99.29

Table V. Concentration of calibration solution of campesterol and the accuracy of the determination

Concentration level	Campesterol		
	Standard concentration (ng/ml)	Concentration found (ng/ml)	Accuracy (%)
1	59.00	60.58	102.68
2	117.00	111.11	94.97
3	234.00	235.06	100.45
4	468.00	491.72	105.07
5	936.00	912.98	97.54
6	1872.00	1761.14	94.08
7	2808.00	3046.94	108.51

Table VI. Concentration of calibration solutions of stigmasterol and their accuracy of determination

Concentration level	Stigmasterol		
	Standard concentration (ng/ml)	Concentration found (ng/ml)	Accuracy (%)
1	136.00	144.6409	106.35
2	272.00	241.2552	88.70
3	544.00	535.5297	98.44
4	1088.00	1152.767	105.95
5	2176.00	2294.101	105.43
6	4352.00	4311.85	99.08
7	6528.00	6656.755	101.97

Table VII. Concentration of calibration solutions of sitosterol and the accuracy of their determination

Concentration level	Sitosterol		
	standard concentration (ng/mL)	Concentration found (ng/mL)	Accuracy (%)
1	132.00	139.48	105.67
2	264.00	244.58	92.65
3	528.00	528.91	100.17
4	1056.00	1094.38	103.63
5	2112.00	1943.13	92.00
6	4224.00	4250.72	100.63
7	6336.00	6996.62	110.43

A chromatogram of the 5 analyzed sterols in the above conditions, is shown in Figure 3.

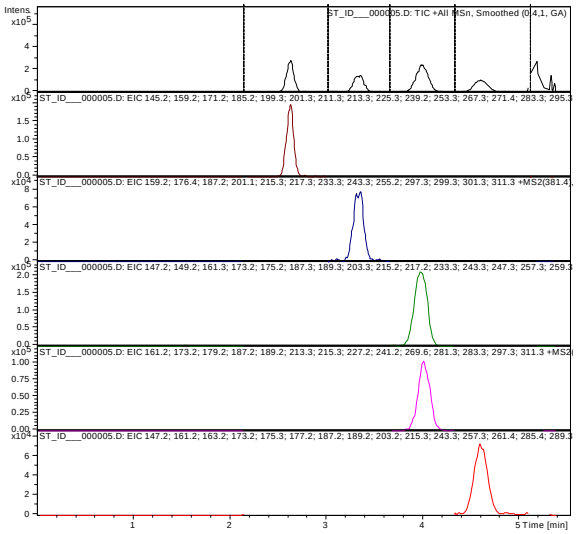

Fig. 3. Chromatograms of the analysed sterols (top to bottom, superimposed signals of standards. a. ergosterol, b. brassicasterol, c. campesterol, d. stigmasterol, e. sitosterol.

Calibration curves of each sterol, on the basis of which the quantification was made are presented in Figure 4 (a-e).

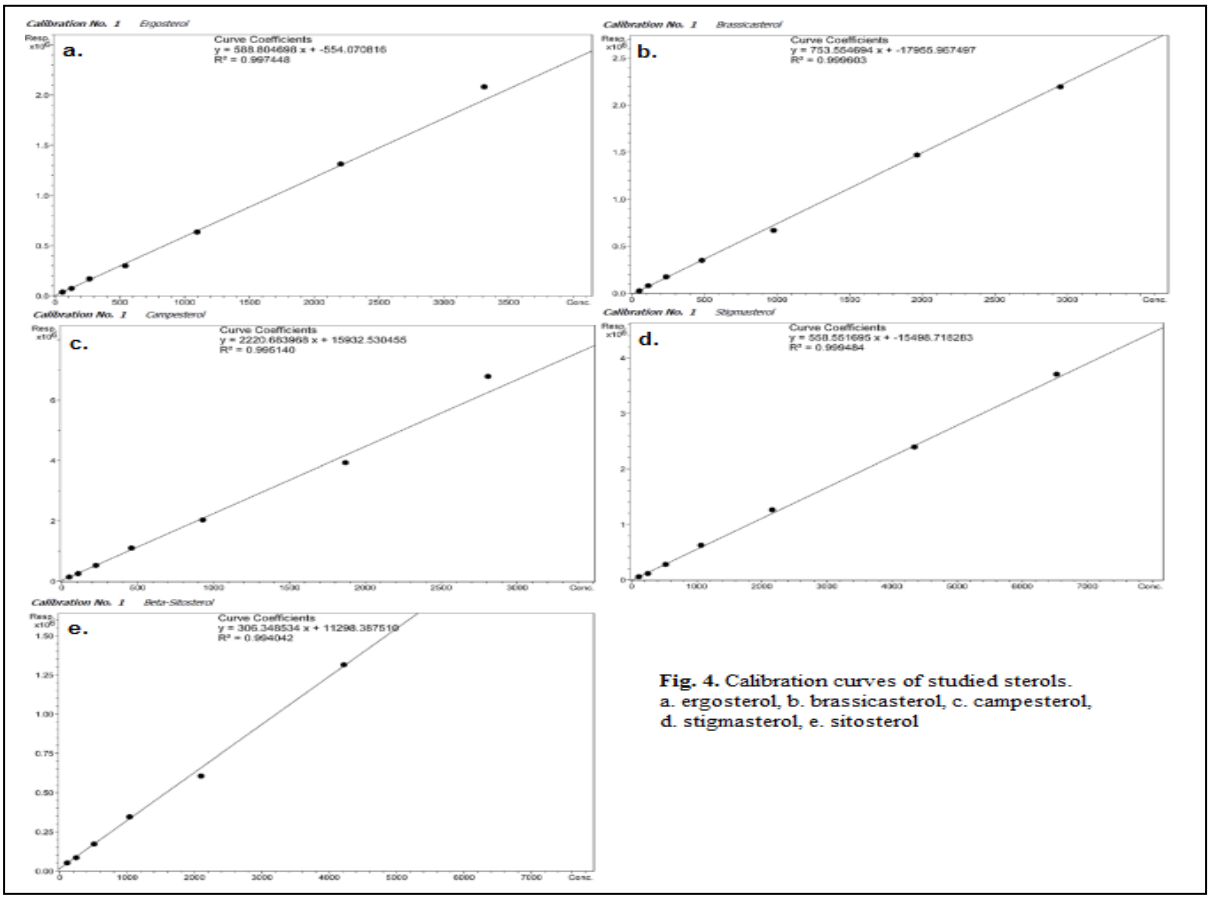

Fig. 4. Calibration curves of studied sterols.
a. ergosterol, b. brassicasterol, c. campesterol, d. stigmasterol, e. sitosterol

RESULTS AND DISCUSSION

The concentrations of the sterols found are presented in Table VIII. Concentrations according to the dilutions carried out are expressed in μg/mL.

Table VIII. Sterol concentrations from analyzed sterols

Sample	Ergosterol	Stigmasterol	Beta-Sitosterol	Campesterol	Brassicasterol
	Concentration (μg/ml)				
X1	0.000	0.000	15.859	0.000	0.000
X2	0.000	0.000	17.251	0.000	0.000
X3	0.000	0.000	21.598	0.000	0.000
X4	0.000	0.000	1.516	0.000	0.000
X5	0.000	0.000	2.033	0.000	0.000
X6	0.000	0.000	0.000	0.000	0.000

Key: X1= RC DH1, X2=BP DH1 , X3=RN DH1, X4= P 30%, X5=VL DH1,X6=CB DH1

Of all the analyzed sterols, the only sterol detected was β-sitosterol. With the exception of the gemmotherapeutic product of *Carpinus betulus* (X6), all samples analyzed contain betasitosterol.

The highest concentrations of β-sitosterol are found in the gemmotherapeutic products of the following *Ribes nigrum, Betula pubescens* and *Rosa canina*. Small quantities are found in *Viburnum lantana* and in *propolis tincture*.

CONCLUSIONS

Due to the fact that in our bibliography we didn`t find the presence of -sitosterol, we consider that our research brings a modest contribution to the knowledge of the chemical composition of the gemmotherapeutic products obtained from *Ribes nigrum, Betula pubescens, Rosa canina*.and *Carpinus betulus*.

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ANALIZA COMPUSILOR STEROLICI DIN EXTRACTELE VEGETALE DE RIBES NIGRUM, ROSA CANINA, BETULA PUBESCENS, CARPINUS BETULUS, VIBURNUM LANTANA SI TINCTURA DE PROPOLIS

REZUMAT

Extractele vegetale de tip gemoterapic in dilutie DH1 de *Rosa canina* (X1), *Betula pubescens* (X2), *Ribes nigrum* (X3), *Viburnum lantana* (X5), *Carpinus betulus* (X6) și tinctura 30% de propolis (X4) sunt frecvent folosite în medicina integrativă pentru acțiune antiinflamatoare. Intrucat efectul antiinflamator al unui extract vegetal poate fi imprimat și de steroli, ne-am propus analiza acestora produse (de proveniență Plantextract, Cluj), prin cromatografie de lichide de înalta performanță cuplată cu spectrometrie de masa (LC/MS). S-au analizat: betasitosterolul, stigmasterolul, campesterolul, ergosterolul, brassicasterolul. Concentratiile de steroli gasite, prin metoda mai sus mentionata (exprimate in µg/mL) au fost semnificative doar pentru betasitosterol, în: X1 = 15.859, X2 = 17.251, X3 = 21.598, X4 = 1.516; X5 = 2.033, X6 neidentificabil.

Cuvinte cheie: extracte vegetale de tip gemoterapic, steroli si propolis, analiza LC/MS/MS

ANTIBACTERIAL EFFECTS OF SOME PLANT EXTRACTS ON *STAPHYLOCOCCCUS AUREUS* STRAINS

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ABSTRACT
Background & Aims: The purpose of this study was to investigate the antibacterial properties of alcoholic extracts and essential oils derived from plants against *S. aureus* strains isolated from patients.
Material and Methods: We studied 50 strains of *S. aureus* isolated from various clinics in Cluj-Napoca, as well as from outpatients and six natural products: Calendula tincture (*Calllendulae flos*), tincture of hawthorn (*Folium Crataegi as flowers*), *Melissa officinalis* essential oil, tincture of sage (*Salviae folium*), propolis tincture and Biosept forte containing 80% thyme essential oil (*Thyme aetheroleum*) and 20% cloves essential oil (*Caryophyllorum aetheroleum*). For each strain susceptibility testing was performed using disk diffusion method on subculture standardized Kirby-Bauer with manual reading.
Results: MSSA strains showed inhibition zone diameters of ≥ 10 mm for *Melissa officinalis* oil at 100% of strains, for propolis tincture in proportion of 93.33% of strains, for Salviae folium at a rate of 86.66% of strains and in 46.66% of strains for both calendula tincture and for hawthorn. MRSA strains showed inhibition zone diameters of ≥ 10 mm, in case of *Melissa officinalis* oil and propolis tincture to 100% of strains, for *Salviae folium* in the proportion of 97.14% of strains, a rate of 28.57% of strains for tincture of hawthorn and the rate of 14.28% of strains for Calendula tincture.
Conclusion: All six plant extracts tested strains of *S. aureus* had antibacterial effects. The best antibacterial effects was observed in case of Biosept forte product. Data showed significant antibacterial effect of *Melissa officinalis* essential oil and propolis. Moderate antibacterial effect was observed for sage tincture (*Salviae folium*), hawthorn (*Folium crataegi*) and marigold (*Calllendulae flos*).
Key words: plant extracts, MRSA, antibacterial effect.

INTRODUCTION

Despite the fact that the pharmaceutical industry produce new antibiotics, in recent years the resistance to chemotherapy is growing. Therefore, there are serious questions over the future use of chemotherapy, which requires taking measures to identify new antibacterial substances.
Currently are evaluated various plant extracts for possible antibacterial effects which covers many bacterial species, including those responsible for causing nosocomial infections, as *Staphylococccus aureus (S. aureus)*.
The evaluation of antimicrobial activity is based on quantification of bacterial development after the contact with natural extracts.
The purpose of this study was to investigate the antibacterial properties of alcoholic extracts and essential oils derived from plants against *S. aureus* strains isolated from patients.

MATERIALS AND METHODS

We studied 50 strains of *S. aureus* isolated from various clinics in Cluj-Napoca, as well as from outpatients.
The identification of isolates was based on morphological characters, cultural, biochemical and antigenic tests. For each strain susceptibility testing was performed using disk diffusion method on subculture standardized Kirby-Bauer with manual reading (according to CLSI).
The natural products tested were: Calendula tincture (*Calllendulae flos*) from Hofigal, tincture of hawthorn (*Folium Crataegi* as flowers) from Fares, *Melissa officinalis* essential oil from Fares, tincture of sage (*Salviae folium*) from Fares, propolis tincture and Biosept forte (natural antibiotic) from the company Fares containing 80% thyme essential oil (thyme aetheroleum) and 20% cloves essential oil (*Caryophyllorum aetheroleum*). In vitro antibacterial activity of the natural products was tested on solid culture media (Mueller Hinton) distributed in Petri dishes (90 mm).

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The preparation of inoculum was made by suspending the colonies in 3 ml saline to the density of a McFarland 0.5 turbidity standard. We used several morphologically similar colonies. The inoculation of the agar plates was made with a sterile cotton swab by removing the excess fluid and by spreading the inoculum over the entire surface of the plate.

After 15 minutes we applied the sterile disks of 5 mm diameter at a distance of 20-25 mm between two neighboring disks. The disks were impregnated with 20µl of each plant extract. We worked with pairs samples, for each strain 2 tests were performed with the same substance. Reading was done after incubation (for 18-20 hours at 37 ˚C) with a ruler measuring the diameter of the inhibition zones (in mm). We noted the mean (arithmetic average) of the two determinations. We started from the principle that bacteria are more sensitive to the substance studied as the inhibition zone diameter is larger.

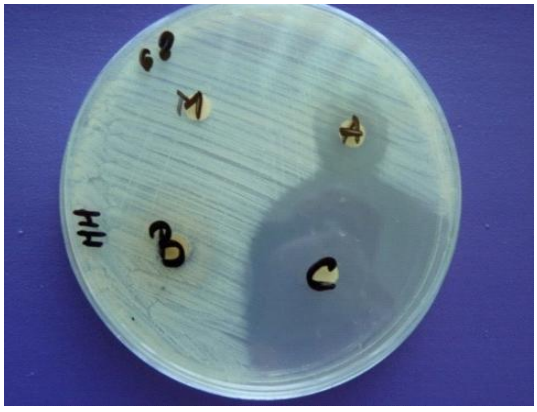

Fig. 1. Effects of natural extracts: A (*Calllendulae flos*), B (tincture of hawthorn) (*Folium crataegi* as flowers) and C (*Melissa officinalis* essential oil) on a MSSA strain

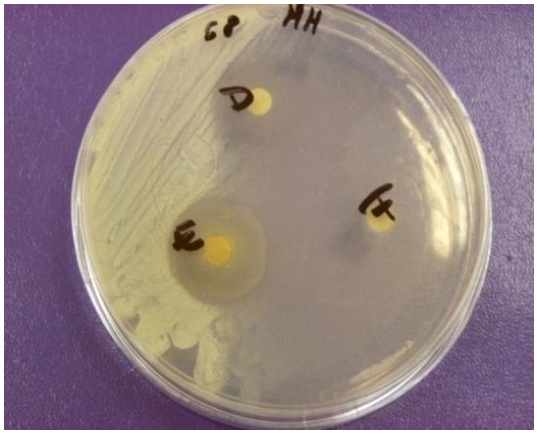

Fig. 2. Effects of natural extracts: D (tincture of sage), E (propolis tincture) and F (Biosept forte) on a MSSA strain

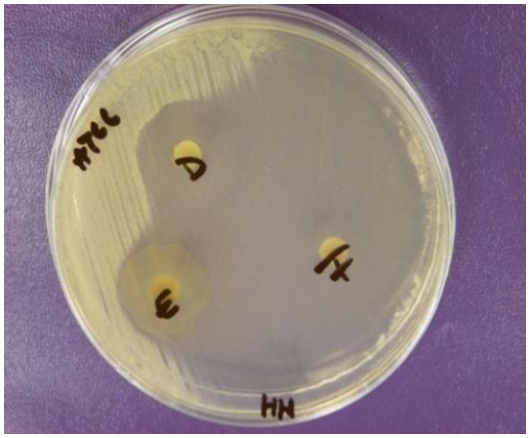

Fig. 3. Effects of natural extracts D (tincture of sage), E (propolis tincture) and F (Biosept forte) on *S. aureus* strain ATCC 25923

Statistical methods. To describe qualitative variables we used absolute and relative frequencies plotted by sector and column charts. In case of quantitative variables we used the average as centrality parameter and standard deviation as the dispersion parameter.

RESULTS

In case of MSSA strains, using the tincture of calendula (*Calllendulae flos*), we obtained diameters of the inhibition zones <10 mm for 18% of strains (9 strains) and in case of MRSA strains we recorded values <10 mm were in 62% of strains (31 strains). The inhibition zone diameters between 11 and 20 mm were recorded in 12% of MSSA (6 strains) and 8% MRSA (4 strains) (Figure 4).

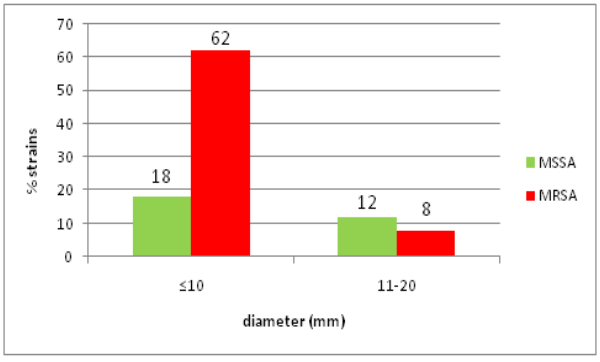

Fig. 4. Diameters of the inhibition zones for Calendula tincture

From 50 strains studied (15 strains MSSA and 35 MRSA strains), 12 strains (24%) showed inhibition zone diameter ≥ 10 mm (7 MSSA strains (14%) and 5 MRSA strains (10%)), of which 4 MSSA strains (8%) showed inhibition zone diameter ≥ 15 mm (Table I).

Table I. Diameters of the zones of inhibition ≥ 10 mm for Calendula tincture

Inhibition zone diameter ≥ 10 mm *Calendula* tincture	MSSA	MRSA	TOTAL %
18	1 (2%)		1(2%)
17	1 (2%)		1(2%)
16	1 (2%)		1(2%)
15	1 (2%)		1(2%)
14	1 (2%)	2 (4%)	3 (6%)
13	-	1 (2%)	1(2%)
11	1 (2%)	1 (2%)	2 (4%)
10	1 (2%)	1 (2%)	2 (4%)
Total	7 (14%)	5 (10%)	12 (24%)

We recorded 38 strains (76%) who had the inhibition zone diameters <10 mm, of which 8 MSSA strains (16%) and 30 MRSA strains (60%) (Table II).

Table II. Diameters of the inhibition zones <10 mm for *Calendula* tincture

Inhibition zone diameter <10 mm Calendula tincture	MSSA	MRSA	TOTAL
Total	8 (16%)	30 (60%)	38 (76%)

Calendula tincture (*Calllendulae flos*) had no effect on 13 strains (26%): 9 MRSA strains (18%) and 4 MSSA strains (8%). Thus, Calendula tincture showed antibacterial effect of the 12 strains (24%) of the 50 strains tested. *Calendula* tincture (*Calllendulae flos*) had no effect on the 38 strains (76%), 30 MRSA strains (60%) and 8 MSSA strains (16%). For hawthorn tincture (*Folium crataegi*) the values of the diameters of the inhibition zones for MSSA were less than 10 mm in 18% of strains (9 strains) and in in 60% of MRSA strains (30 strains). The diameters of the of inhibition zones were between 11 and 20 mm in 12% of MSSA strains (6 strains) and in 10% of MRSA strains (5 strains) (Figure 5).

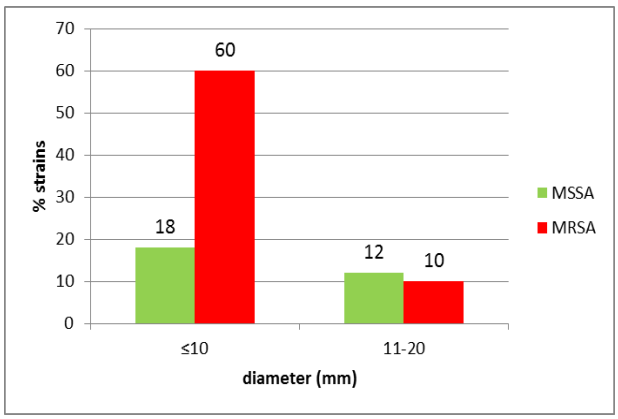

Fig. 5. Diameters of the inhibition zones for hawthorn tincture

From all studied strains, 17 strains (34%) showed an inhibition zone diameter ≥ 10 mm: 7 MSSA strains (14%) and 10 MRSA strains (20%). 3 MSSA strains (6%) showed inhibition zone diameters ≥ 15 mm (Table III).

Table III. Diameters of the inhibition zones ≥ 10 mm for Hawthorn tincture

Inhibition zone diameter ≥ 10 mm Hawthorn tincture (***Folium crataegi***)	**MSSA**	**MRSA**	**TOTAL**
18	-		-
17	1 (2%)		1 (2%)
16	1 (2%)		-
15	1 (2%)		1(2%)
14	2 (4%)	4 (6%)	6 (12%)
13	1 (2%)	1 (2%)	2(4%)
12	-	-	
11	-	-	-
10	1 (2%)	5 (10%)	6 (12%)
Total	7 (14%)	10 (20%)	17 (34%)

Thus, the hawthorn tincture showed antibacterial effect on 17 strains (34%) of the 50 tested strains and had no effect on 33 strains(66%): 25 MRSA strains (50%) and 8 MSSA strains (16%).

For *Melissa officinalis* essential oil, the diameters of the inhibition zones for MSSA ranged from 21-40 mm in 12% of strains (6 strains) and they were less than 20 mm in 18% of strains (9 strains). In case of MRSA inhibition zone diameter values were less than 20 mm in 64% of strains (32 strains) and were between 21 and 40 mm in 6 strains (Figure 6).

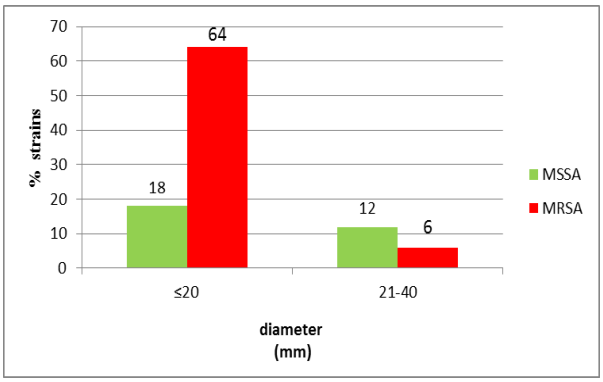

Fig. 6. Diameters of the inhibition zones for *Melissa officinalis* essential oil

In case of sage tincture (*Salviae folium*), the diameters of the inhibition zones were for MSSA strains between 9 and 27 mm, and for MRSA strains they were between 8-18 mm. 9 strains were resistant. Diameters ranging from 15 to 18 mm have been reported to 6 MSSA strains (12%) and to 14 MRSA strains (28%) (Figure 7).

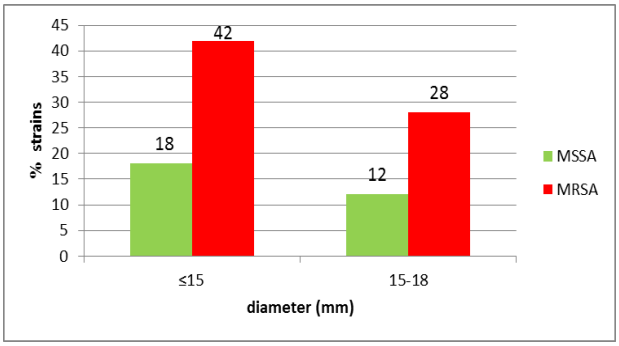

Fig. 7. Diameters of the inhibition zones for sage tincture

For propolis tincture, the diameters of the inhibition zones for MSSA strains were between 9-26 mm. For MRSA strains, the diameters were between 16 and 24 mm. Diameters between 21 and 26 mm have been reported for 7 strains of MRSA (14%) and 6 MSSA strains (12%) (Figure 8).

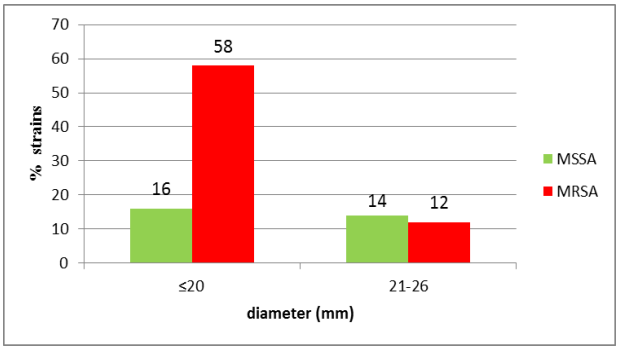

Fig. 8. Diameters of the inhibition zones for propolis tincture

In case of Biosept forte, the diameters of the zones of inhibition for MSSA were between 51-61 mm. For MRSA the diameters of the zones of inhibition ranged between 40-65 mm. We reported diameters larger than 51 mm at 12 MSSA strains (24%) and at 28 MRSA strains (56%) (Figure 9).

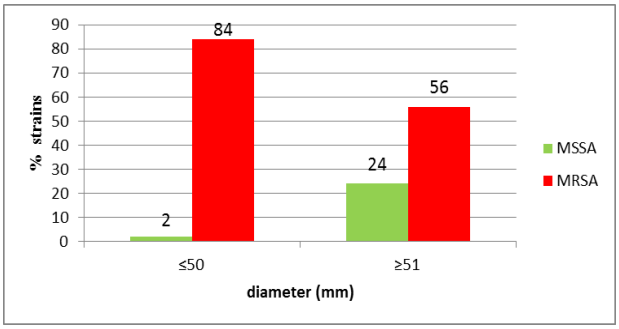

Fig. 9. Diameters of the zones of inhibition for Biosept forte

MSSA strains showed inhibition zone diameters of ≥ 10 mm for *Melissa officinalis* oil at 100% of strains, for propolis tincture in proportion of 93,33% of strains, for *Salviae folium* at a rate of 86,66% of strains and in 46,66% of strains for both *Calendula* tincture and for hawthorn.

MRSA strains showed inhibition zone diameters of ≥ 10 mm, in case of *Melissa officinalis* oil and propolis tincture to 100% of strains, for *Salviae folium* in the proportion of 97,14% of strains, a rate of 28,57% of strains for tincture of hawthorn and the rate of 14,28% of strains for *Calendula* tincture (Figure 10).

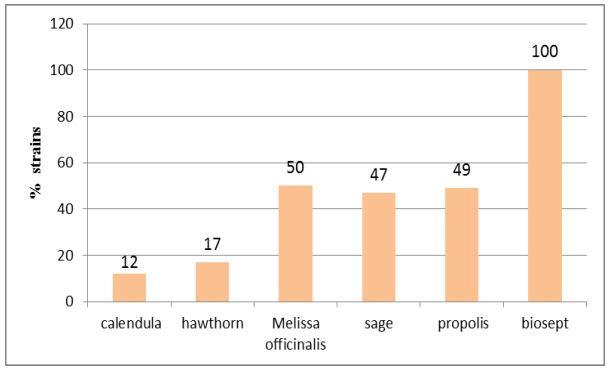

Fig. 10. Diameters of the inhibition zones ≥ 10 mm for MSSA and MRSA strains

DISCUSSION

Given the alarming rise of antibiotic resistance, there is a growing concern among researchers for the discovery of new antibacterial substances. Thus, the study of antibacterial effects of plant extracts is very important. The use of plants in therapy has many advantages, including: low cost and low toxicity when administered at high doses.

Plants contain components with antibacterial activity whose use is increasing worldwide. Antioxidants are considered protective agent, serving to enhance the oxidative processes. The components with antioxidant effects are phenolic compounds, especially flavonoids, which interact with free radicals. Antibacterial effect could be the result of induction of free radicals [6,10].

In our study, the most intense antimicrobial product can be considered Biosept forte, which presented inhibition zone diameters of 65 mm maximum, followed by essential oil of *Melissa officinalis* with maximum 40 mm inhibition zone diameters.

Propolis tincture had also an important antimicrobial effect, for which the maximum diameter of the inhibition zones was 26 mm and 28% from all strains presented a diameter between 21 and 26 mm.

In case of these 50 strains tested strains, the inhibition zone diameters for sage tincture ranged between 15 and 18 mm for 40% of strains, showing an antimicrobial effect.

Hawthorn tincture (*Folium crataegi*) had no effect on 78% of *S. aureus* strains tested, and in case of marigold tincture (*Calllendulae flos*), 80% of strains were found to be resistant.

Based on these diameters we can conclude that most plant extracts showed antibacterial effect.

A recent study in Brazil reveals anti-inflammatory, antibacterial and angiogenic properties of *C. officinalis* [14].

A study based on several aerobic and facultative anaerobic bacterial species including S. aureus indicates a moderate inhibitory activity with MIC> 2048 mg / L in all bacteria tested, except *Prevotella spp* [9].

In Portugal, a study on several herbs highlights in case of *Crataegus monogyna* a highest concentration of phenolic acids (5.5 mg/gdw), which proves an important antioxidant activity [2].

Melissa officinalis has sedative [20], antiviral and antibacterial effects [5]. Good antimicrobial effects were recorded on a multidrug-resistant strains of *Shigella sonei*.

Antimicrobial effects of sage is due to its components: oleanolic acid, triterpenoid and were identified MRSA and *Streptococcus pneumoniae* [8,22].

A study on *Salvia corrugator Vahl* identified antibacterial activity on Gram-positive bacteria of two components: fruticuline A and demetilfruticuline. The first presented bacteriostatic activity on all tested strains and the two showed significant bactericidal activity against *S. aureus* and *S. epidermidis* and bacteriostatic activity on *Enterococcus faecalis* and *Enterococcus faecium* [4].

A study made in Tunisia shows antimicrobial and antioxidant effect of methanolic extracts of sage [16], while another study in Jordan on several plant extracts, including sage showed no antibacterial effect on *S. aureus* and *P. aeruginosa* [1].

Takaisi & Schilcher have suggested that the antimicrobial action of propolis is due to the combined action of some constituents that inhibit the bacterial RNA polymerase (pinocembrine, galangine and caffeic acid–phenethyl-ester) [21].

There are studies that show the antibacterial effect of propolis on MRSA [3,15], it is higher than the antibacterial effect of honey [13] and more pronounced on Gram-positive [18] bacteria compared to gram-negative bacteria and fungi [19]. Propolis increases the antibacterial effect of antibiotics such as Ampicillin, Gentamicin, Netilmicin, Chloramphenicol, Vancomycin [7] and Streptomycin [17]. The antibacterial effect against *S. aureus* was evidentiated in Japan, but data obtained emphasises its variation depending on the area and the month in which this was collected [12].

The essential oil of *Thymus vulgaris* showed an antibacterial effect on *E. coli* [11].

CONCLUSIONS

- All six plant extracts tested strains of *S. aureus* had antibacterial effects;
- The best antibacterial effects was observed in case of Biosept forte product;
- Data showed significant antibacterial effect of *Melissa officinalis* essential oil and propolis;
- Moderate antibacterial effect was observed for sage tincture (*Salviae folium*), hawthorn (*Folium crataegi*) and marigold (*Calllendulae flos*).

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EFECTELE ANTIBACTERIENE ALE UNOR EXTRACTE DIN PLANTE ASUPRA TULPINILOR DE *STAPHYLOCOCCCUS AUREUS*

REZUMAT

Scop: Scopul acestui studiu a fost de a investiga proprietatile antibacteriene ale extractelor alcolice si uleiurilor esentiale derivate din plante asupra tulpinilor de *S. aureus* isolate de la pacienti.

Materiale si metode: Am studiat 50 de tulpini de *S. aureus* isolate din diferite institutii spitalicesti din Cluj-Napoca, precum si de la pacienti tratati ambulator, si 6 produse naturale: tinctura de galbenele (*Calllendulae flos*), tinctura de paducel (*Folium Crataegi as flowers*), ulei esential de *Melissa officinalis*, tinctura de salvie (*Salviae folium*), tinctura de propolis si Biosept forte, care contine 80% ulei esential de cimbru (*Thyme aetheroleum*) si 20% ulei esential de cuisoare (*Caryophyllorum aetheroleum*). Pentru fiecare tulpina, testarea suscetibilitatii a fost efectuata folosind metoda difuzimetrica standardizata a subculturilor Kirby-Bauer cu citire manuala.

Rezultate: Tulpinile de MSSA au prezentat zone de inhibitie cu diametrul ≥ 10 mm la uleiul de *Melissa officinalis* pentru 100% dintre tulpini, la tinctura de propolis in proportie de 93,33% dintre tulpini, la *Salviae folium* 86,66% dintre tulpini si 46,66% dintre tulpini la tinctura de galbenele si paducel. Tulpinile de MRSA au prezentat zone de inhibitie cu diametrul ≥ 10 mm in cazul uleiului de *Melissa officinalis* si a tincturii de propolis pentru 100% dintre tulpini, 97,14% tulpini pentru *Salviae folium*, 28,57% tulpini pentru tinctura paducel si 14,28% tulpini pentru tinctura de galbenele.

Concluzii: Toate cele sase extracte din plante testate pe tulpinile de *S. aureus* au prezentat efecte antibacteriene. Cele mai bune efecte antibacteriene au fost observate in cazul produsului Biosept forte. Datele arata un effect antibacterian semnificativ al uleiului esential de *Melissa officinalis* si propolis. Efect antibacterian moderat a fost observat in cazul tincturii de salvie (*Salviae folium*), paducel (*Folium crataegi*) si galbenele (*Calllendulae flos*).

Cuvinte cheie: extracte din plante, plant extracts, MRSA, efect antibacterian.

ACUTE ORAL AND DERMAL EFFECTS OF SPIRULINA IN MICE

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ABSTRACT

Spirulina (SP) is a spiral-shaped, blue-green microalga included in bacteria kingdom. SP has a rich content in essential amino acids, phycobiliproteins (phycoerythrin, phycocyanin), minerals, vitamins, carotenoids, polyphenols and essential fatty acids. The aim of this study consists in a safety evaluation of SP treatment using oral and dermal toxicity studies. SP has revealed no significant changes in skin parameters, behavior or other locomotion problems when was administered at limited doses of 2000 mg/bw/24 h and 5000 mg/bw/24h on SKH1 mice. Consequently, SP is a natural product that can be used both by adults and children, being characterized by multi-target beneficial effects for all whole human body and, nevertheless, by lack of toxicity.

Keywords: spirulina, acute toxicity, skin toxicity, subchronic toxicity, oral toxicity

INTRODUCTION

Spirulina (SP) is a spiral-shaped, blue-green alga [1] which belongs to the *Oscillateriaceae family* [2]. Firstly, SP was included in the plant kingdom, although, in the present it belongs to the bacteria kingdom because of its biochemical, physiological and genetics properties [3]. The most commonly used species of SP are: *Arthrospira platensis*, *Arthrospira maxima* and *Arthrospira fusiformis* [3,4]. SP has a rich content in essential amino acids, phycobiliproteins (red phycoerythrin, blue phycocyanin) [5], minerals, vitamins (B_1, B_2, B_3, B_5, B_6, B_7, B_9, B_{12}, D, E), carotenoids (alpha-carotene, beta-carotene, zeaxanthin) [6,7] and essential fatty acids (3,6 gamma-linolenic acid, linoleic acid, stearidonic acid and arachidonic acid) [8-12]. The methanolic extract of SP has also revealed high amounts of phenolic compounds including salicylic, trans-cinnamic, chlorogenic and caffeic acids that have been responsible for its antioxidant effects [13].

Furthermore, the microelements of SP depend both on the type and the mineral pollution [14] of producing areas [15]. SP and its derivatives are widely used for their antioxidant, anticancer, anti-inflammatory, anti-diabetic, antimicrobial, as well as for their immunostimulant effects [16-18]. In this regard, SP is generally recommended in various chronic pathologies including arterial hypertension, hypercholesterolemia, malnutrition, obesity, sugar diabetes and cancer [19,20]. SP is considered to be one of the most complex natural products that is widely used not only by adults but also by children [21]. The aim of this study consists in a safety evaluation of SP treatment using oral and dermal toxicity studies.

MATERIALS AND METHODS

This experimental study was done on healthy young adult SKH1 mice. The females were non-pregnant. All the animals involved in the study were between 8 and 12 weeks old. The mice were housed in standard conditions: room temperature 22ºC (+ 3ºC), relative humidity between 30% and 70 % and artificial lighting characterized by 12 hours of light and 12 hours dark. During the experiment the mice have received food consisting in special pellets and water *ad libitum*. The animals were randomly selected, marked for individual identification and acclimated to the laboratory conditions for 5 days before to begin the experiment. The animals that were included in the study have appropriate size and age [22].

Spirulina, a dark blue-green powder was purchased from FAVISAN Laboratories, Lugoj, Romania.

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Acute toxicity studies

Hairless SKH1 mice weighing 25 – 30 g were included in the study. Acute oral and dermal toxicities studied were performed according to OECD- 425 and OECD-434 guidelines. Because SP extract is widely used for centuries all around the world with no major side effects we concluded to apply the limit test for evaluating the acute oral and dermal toxicity of this natural product. According to OECD-425, only one mouse was firstly selected for the acute oral study. It was fasted overnight and in the next morning it was dosed with a test dose of 2000 mg/bw/24h. The animal has survived, and we continued to dose other four female mice with the same dose of SP. The animals have also survived. After that, we continued to give a dose of 5000 mg/bw/24 h to one mouse firstly and after that to the other three mice. The animals were observed for side effects for 14 days and after that were sacrificed.

According to OECD-434, for acute dermal toxicity test were selected 18 mice having approximately the same age and weight. These mice were divided in 4 groups: 5 female mice (group 1), 5 male mice (group 2), 4 healthy mice treated with diluted solution of alcohol (2 males, 2 females) (group 3), 4 healthy untreated mice (2 males, 2 females). SP was dissolved in a diluted alcoholic solution (67%) and, then, was applied uniformly over 10 % of the total body surface area. The skin parameters (melanin levels and erythema) were decelated using a mexameter MX18 (Courage& Khazaka Electronics, Germany) and the skin hydratation was evaluated using a corneometer CM825 MX18 (Courage&Khazaka Electronics, Germany). All animals were observed for 14 days after application of SP extract and after that were sacrificed.

The experimental study protocol was designed according to the Universal Declaration of Animal Rights proclaimed in Paris in 1978 and to the Declaration of Helsinki, the European Convention (ETS No. 123) amended in 1998 (ETS No. 170) and Council Directive 86/609/EEC on the protection of vertebrate animals used for experimental and other scientific purposes. The animal study protocol was approved by the Ethics Committee of the Faculty of Pharmacy, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania.

RESULTS

It was observed that all the mice that received SP in both doses of 2000 mg/bw/24 h and 5000 mg/bw/24 h have survived and they did not manifest any behavioral changes, convulsions, locomotion problems or mortality (Figure 1). Consequently, the LD50 for SP was greater than 5000mg/bw/24h.

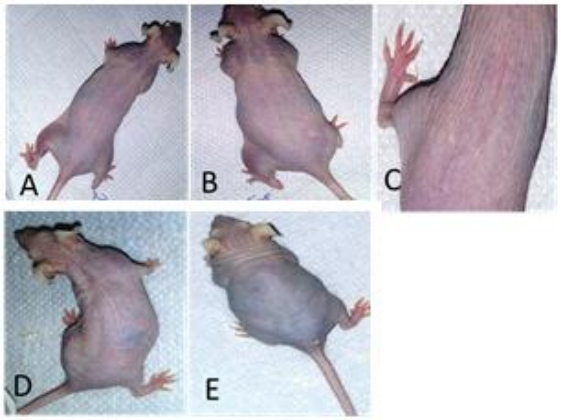

Fig. 1. Mice – 24 hours after the skin application of SP extract: A) male mice treated with alcoholic solution of SP; B) female mice treated with alcoholic solution of SP; C) skin of female mice treated with alcoholic solution of SP; D) female mice treated with alcoholic solution; E) female mice untreated

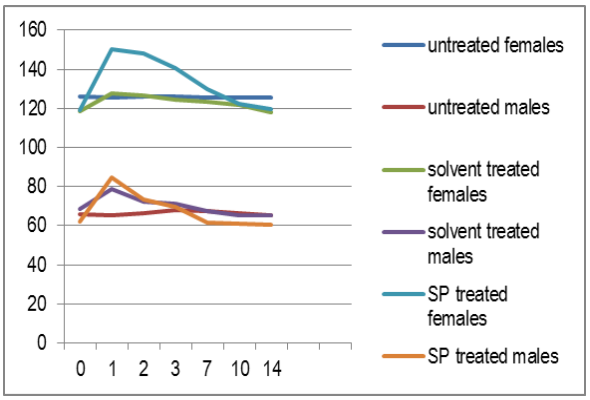

Fig. 2. Evolution of melanin levels in mice

There were no significant differences between the values of melanin before and after SP application in female mice (p = 0.2097) p>0.05 and in male mice (p = 0.7517) p>0.05.

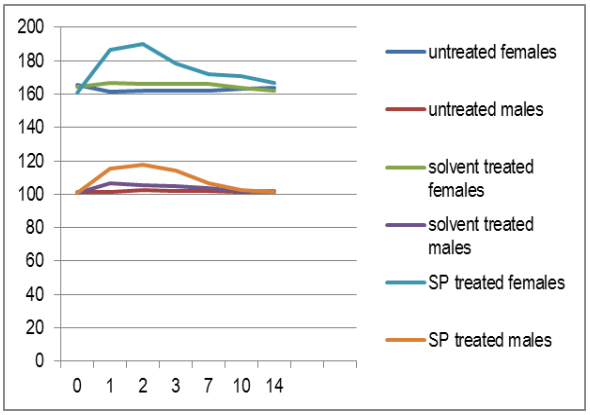

Fig. 3. Evolution of erythema in mice

The erythema values increased in the first 48 h after local administration of SP both in female ($p = 0.035$) $p<0.05$ and male mice ($p = 0.05222$) $p>0.05$ but significant changes were seen only in female mice treated with SP. The alcoholic solution that was used as solvent did not caused significant differences in female ($p = 0.1000$) $p>0.05$ and in male groups ($p = 0.071$) $p>0.05$. Consequently, the significant changes in females group treated with SP were caused only by SP and not by the solvent.

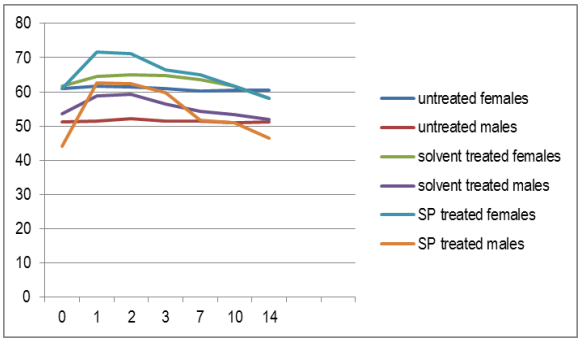

Fig. 4. Evolution of skin hydratation levels in mice

There were no significant differences regarding the skin hydratation measurements before and after the local application of SP in female ($p = 0.060$) and in male group ($p = 0.3830$), both $p > 0.05$. Consequently, according to our toxicity studies on mice, we concluded that SP has lack oral and dermal toxicity, because the majority of the skin parameters that were measured after the local administration of SP extract did not suffer significant changes. The erythema levels were significantly higher only in the female group treated with SP. However, the information regarding the side effects of SP treatments is still limited. According to another experimental study, higher dose of SP (1000 mg/kg) did not cause important changes in cardiac parameters in mice [23].

Some clinical trials tested the toxicity of SP in humans. There have been reported only minor hypersensitivity reactions including urticarial, rash, diaphoresis and bronchospasm [24]. Other side effects observed in patients treated daily with 1g of SP were: flushing of the face, headache, muscle pain, sweating and difficulty concentrating [25]. Moreover, it was reported a higher rate of protidemia and creatinemia in HIV patients treated with SP for 6 month [26]. It has been reported a clinical case of an 82-year-old healthy woman who manifested bullae, partly hemorrhagic, on the trunk and extremities, secreting erosions and submammary macerations after a 2-year period of SP intake. Another 57-year-old man diagnosed with pemphigus vulgaris has developed a severe flare-up of the disease after consumption of a supplements containing Ginseng, Ginkgo biloba and SP. Another case of a 45-year-old woman who presented dermatomyositis after intake of a supplement based on methylsulfonylmethane, organic cayenne pepper, the algae Aphanizomenon flos-aquae and SP was described [27]. Moreover, SP caused anaphylaxis to a 17-year-old male after one ingestion of a SP tablet [28] and acute rhabdomyolysis to another patient which consumed a dietary supplement containing SP [29].

According to Health Food Organization, 4g of SP is daily recommended for an adult of 50 kg body weight [30].The beneficial effects of SP are recognized by World Health Organization (WHO), Food and Agriculture Organization of the United Nations, United Nations Industrial Development Organization and United Nations Children's Fund [2]. The WHO projects considered SP to be one of the most curative and prophylactic components of nutrition in the 21st century [31] due to its remarkable nutrient profile, lack of toxicity and therapeutic effects [32]. The Dietary Supplements Information Expert Committee of the United States Pharmacopeia Convention assigned a class A safety rating for SP *maxima* and SP *platensis*, including these dietary supplement ingredients as monographs in United States Pharmacopeia and National Formulary [33].

CONCLUSION

The acute oral and dermal toxicity studies that were made on hairless SKH1 mice using limit doses of 2000 mg/bw/24 h and 5000 mg/bw/24 h of SP revealed no significant changes in skin parameters, behavior or other locomotion problems. Moreover, all the animals included in study have survived; in female group it has been observed a higher level of erythema parameters. We consider that SP is a complex natural product with multi-target beneficial effects in rats characterized by lack of toxicity that can be used both by adults and children.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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EFECTELE ACUTE ORALE ŞI CUTANATE ALE SPIRULINEI PE MODEL ANIMAL DE ŞOARECE

REZUMAT

Spirulina (SP) este o microalgă albastră-verde, cu formă spiralată, fiind inclusă în regnul bacteriilor. SP are un conţinut bogat în aminoacizi esenţiali, ficobiliproteine (ficoeritrină şi ficocianină), minerale, vitamine, carotenoizi, polifenoli şi acizi graşi esenţiali. Scopul acestui studiu constă în evaluarea siguranţei tratamentului cu SP prin aplicarea unor texte de toxicitate acută orală şi cutanată. În urma tratamentului oral şi local cu SP utilizându-se doze limită de 2000 mg/kg corp/24h şi 5000 mg/kg corp/24 h nu s-au observat modificări semnificative în ceea ce priveşte parametrii studiaţi, şi comportamentul animalelor şi nu au fost semnalate alte probleme de mobilitate. În concluzie, SP este un produs natural care poate fi utilizat în siguranţă atât de adulţi cât şi de copii, având numeroase efecte benefice asupra organismului şi nu in ultimul rând datorită toxicităţii sale foarte reduse.

Cuvinte cheie: spirulina, toxicitate acută, toxicitate cutanată, toxicitate subcronică, toxicitate orală

THE ROLE OF *SPIRULINA PLATENSIS* IN THE CONTROL OF TYPE 2 DIABETES MELLITUS

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ABSTRACT

In recent years, many experimental and clinical studies evaluated blue-alga *Spirulina platensis* for the treatment of diabetes mellitus (DM). The objective of this study was to evaluate the effects of Spirulina supplements in the control of type 2 diabetes mellitus. The study included patients aged 30-70 years with type 2 DM for at least two years and that have been treated with a stable dose of Metformin for at least 3 months. This randomized, single blinded on parallel groups study included 30 patients with DM type 2, divided in two groups: 15 patients treated with Metformin and placebo (group 1) and 15 patients treated with Metformin and Spirulina (group 2). Spirulina was administered twice per day during 2 months, in a total dose of 800 mg daily. The following parameters were evaluated: blood pressure, total cholesterol (TC), triglycerides (TG), LDL-cholesterol, HDL-cholesterol, fasting serum glucose, and glycated hemoglobin (HbA1c). After 2 months, the mean glycemic value significantly decreased from 124.5±29.28 mg/dl to 108.3±12.53 mg/dl (p<0.003) in group 1 and from 149.5 ±19.89 mg/dl to 121.1± 10.66 mg/dl in group 2 (p<0.001).The mean value of HbA1c significantly decreased from 7.2±0.63% to 6.3 ± 0.69% in group 1 (p=0.008) and from 7.1±0.92% to 6.6 ±0.33 % in group 2 (p=0.009).

Keywords: *Spirulina platensis*, lipids, type 2 diabetes mellitus

INTRODUCTION

Diabetes Mellitus (DM) is a chronic disease that requires long term medical attention to limit and manage the development of devastating complications [1]. An early initiation of pharmacological therapy was associated with improved glycemic control and reduced long term complications in type 2 DM. Many experimental and clinical studies have shown the beneficial effects of food supplements in DM and, particularly, the beneficial effects of *Spirulina platensis* in the control of type 2 DM. Spirulina is a filamentous cyanobacterium worldwide used as a food supplement [2]. *Spirulina platensis* (*Arthrospira platensis*) is one of the three species investigated most often [3]. Spirulina has many nutritional components and it is a rich source of phycocyanin, vitamins, especially vitamin B12 and provitamin A (ß-carotene), minerals, and carotenoids [4]. The lack in cellulose of cell walls in favor of easy digestion is another great advantage of Spirulina consumption [5].

Many experimental and clinical studies have shown the beneficial metabolic, antiviral, liver-protecting, blood-vessel relaxing, anti-cancer, anti-inflammatory and antioxidant properties of Spirulina supplements [6]. In Korea, a double blind, randomized clinical trial evaluated the effects of Spirulina consumption on lipid parameters, immune markers and antioxidant capacity in elderly men. As compared to placebo, Spirulina users had lower lipid parameters, increased serum interleukin-2, but decreased serum interleukin-6, researchers concluding that Spirulina is suitable as a functional food [7]. Another study conducted in Greece showed the beneficial effects of spirulina supplements on lipid profile in 52 dyslipidemic patients. The lipid fractions total cholesterol (TC), triglycerides (TG), LDL-cholesterol (LDL-C) and non-HDL-cholesterol (non-HDL-C) were significantly reduced after 3 months of Spirulina supplementation, the major reduction being observed in TG levels (-16.3%) in women. However this reduction reached 21.3% in women aged > 47 years was 21.3% and 18.6% in women with TG levels >150 mg/dl [8]. These findings are extremely valuable considering the importance of elevated TG levels as a risk factor for cardiovascular disease in women [9]. This study concluded that Spirulina supplementation at a dose of 1g daily has strong hypolipidemic effects especially on TG concentration,

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being proposed as a dietary supplement in dyslipidemic patients [8]. Another study done by Parikh *et al* on type 2 DM patients evaluated the hypolipidemic and hypoglycemic effect of Spirulina [10]. The efficacy of Spirulina supplementation (2g/day for 2 months) in both placebo and Spirulina groups was evaluated by determining serum lipid concentrations, blood glucose levels and HbA1c [10]. Spirulina significantly decreased blood glucose, HbA1c TC, TG and apolipoprotein B levels, while HDL-C and apolipoprotein A1 levels marginally increased. This study concluded that Spirulina supplementation has beneficial effects in controlling glycemia and improving the lipid profile of type 2 DM patients [10].

Despite de evidence, the effects of Spirulina supplementation as add-on therapy in diabetic patients have not been conclusive. The objective of this study was to evaluate the effects of Spirulina supplements on lipid profile and glycemic parameters in patients with type 2 DM.

MATERIAL AND METHODS

This interventional study (800 mg Spirulina/daily) was a randomised, single blinded study on parallel groups with 2 months duration. The patients included in the study were aged 30-70 years, diagnosed with type 2 DM for at least two years and under treatment with a stable dose of Metformin for at least 3 months. The exclusion criteria were BMI > 40 kg/m², any acute or chronic inflammatory disease, cancer, kidney disease, moderate or severe hepatic disease and frequent use of antioxidants and anti-inflammatory. The study included 30 patients with type 2 diabetes mellitus (DM), divided as follows: Group 1: 15 patients with DM type 2 + Metformin+ Placebo and Group 2: 15 patients with DM type 2 + Metformin+ Spirulina supplements.

All subjects were instructed to maintain an isocaloric diet and continue with their eating habits throughout the study. The physical examination, chest radiograph, 12-lead electrocardiogram, anthropometric measurements, blood pressure and laboratory tests was carried out at the beginning and after two months of treatment. A venous blood sample was drawn from an antecubital vein in all subjects after an overnight fast to determine total cholesterol (TC), triglycerides (TG) and HDL-cholesterol and uric acid using standard enzymatic method on a *COBAS Integra 400 plus* analyzer. Bayer diagnostic kit was used to estimate the blood glucose levels. The glycated haemoglobin was determined by the variant haemoglobin A1c using the high performance liquid chromatography technique, using Randox diagnostic kits. Hypertension was diagnosed according to 2013 European Guidelines for the Management of Arterial Hypertension [11]. Diabetes was defined by the current intake of oral hypoglycemic treatment or use of insulin.

For this interventional clinical trial, FAVISAN Company (www.Favisan.ro) provided spirulina and placebo capsules. During this study all subjects were asked to refrain from any source related supplements other than those provided by the study. The compliance of all patients was evaluated weekly by telephone.

Statistical analysis was carried out with the programme SPSS-13, which is a software package used for statistical analysis by comparison of data from two samples, paired t test was used and unpaired t-test was used respectively when appropriate (1 and 2 tail tests).

The study was conducted according to the guidelines for clinical investigation for the treatment and prevention of diabetes CPMP / EWP / 1080 / 00Rev1 delivered on 14 May 2012 by the European Medicines Agency, respecting the Charter of Fundamental Rights of the EU and WMA Declaration of Helsinki (1964) last amended in 2008. This study was conducted in accordance with the Good Clinical Practice of the European Community, CPMP/ICH/135/95 and the ICH Guidelines for Good Clinical Practice: Consolidated Guidance (GCP E-6, April 1996) / and the Declaration of Helsinki and the applicable local laws and regulations.

RESULTS

The study included 30 patients, divided in two groups, similar in terms of age and sex: 15 patients with DM type 2 treated with Metformin and placebo (group 1) and 15 patients with DM type 2 treated with Metformin and the supplement Spirulina twice daily, in a total dose of 800 mg (group 2). No significant differences between anthropometric measurements, mean blood pressure (BP), lipids and glycemic parameters were observed between the two groups at inclusion in the study (Table I).

Table I. Characteristics of the two groups at inclusion in the study (*n*=30)

Studied variables	Group	Mean ±Standard deviation (SD)	*p* value and significance
Age	DM	61.7 ±6.85	0.956^{ns}
	CONTROL	61.6±8.90	
Weigh (kg)	DM	103.1±16.09	0.738^{ns}
	CONTROL	100.7±22.04	
Height (cm)	DM	168.0±7.23	0.686^{ns}
	CONTROL	167.0±5.82	
Body mass index	DM	36.6±6.05	0.861^{ns}
	CONTROL	36.2±6.93	
Waist circumference (cm)	DM	115.3±8.55	0.503^{ns}
	CONTROL	113.1±8.29	
Visceral fat area (cm^2)	DM	195.3±42.97	0.931^{ns}
	CONTROL	194.0±37.45	

Systolic blood pressure (mmHg)	DM	141.9±15.57	0.933^{ns}
	CONTROL	142.4±15.80	
Diastolic blood pressure (mmHg)	DM	82.0±8.82	0.712^{ns}
	CONTROL	83.2±8.68	
Glycemia (mg/dl)	DM	149.5±19.89	0.705^{ns}
	CONTROL	124.5±29.28	
TC (mg/dl)	DM	203.3±64.02	0.266^{ns}
	CONTROL	211.25±66.43	
TG (mg/dl)	DM	191.2±72.28	0.580^{ns}
	CONTROL	198.7±85.09	
HDL (mg/dl)	DZ	43.9±12.68	0.702^{ns}
	CONTROL	45.8±16.37	
LDL (mg/dl)	DM	121.9±51.31	0.259^{ns}
	CONTROL	130.6±46.76	
Uric acid (mg/dl)	DM	6.2±1.73	0.373^{ns}
	CONTROL	6.8±1.84	0.08^{ns}

Key: ^{ns} – non-significant differences (p>0.05)
^{s} – Significant differences (p<0.05)

Table II. The comparison between the parameters studied at inclusion and after two months of Spirulina supplementation in the placebo group (*n*=15) and Spirulina group (*n*=15)

The variables studied	PLACEBO GROUP		SPIRULINA GROUP	
	Mean± Standard deviation	*p* value	Mean± Standard deviation	*p* value
Weight (kg)	100.7 ±22.04	0.008^{s}	103.1 ±16.09	0.001^{s}
Weight after 2 months	96.5±14.2		100.8±14.57	
Glycemia (mg/dl)	124.5±29.28	<0.003^{s}	149.5±19.89	<0.001 ^{s}
Glycemia after 2 months	108.3±12.53		121.1±10.66	
HbA1c %	7.2±0.63	0.008^{s}	7.1±0.92	0.009^{s}
HbA1c after 2 months	6.3±0.69		6.6±0.33	
TC (mg/dl)	211.25±66.43	<0.001^{s}	203.3±64.02	0.004^{s}
TC after 2 months	144.58±38.58		168.5±37.58	
TG (mg/dl)	198.7±85.09	0.02^{s}	191.2±72.28	0.019^{ns}
TG after 2 months	139.9±45.09		154.5±33.71	
HDL (mg/dl)	45.8±16.37	0.04^{s}	43.9±12.68	0.113^{ns}
HDL after 2 months	48.5±15.36		45.5±12.45	
LDL (mg/dl)	130.6±46.76	0.07^{ns}	121.9±51.31	0.059^{ns}
LDL after 2 months	119.4±40.45		99.3±18.98	
Uric acid	6.8±1.84	0.008^{s}	6.2±1.73	0.471^{ns}
Uric acid after 2 months	5.58±0.84		5.04±0.86	

Key: ^{ns} – non-significant differences (*p*>0.05)
^{s} – Significant differences (*p*<0.05)

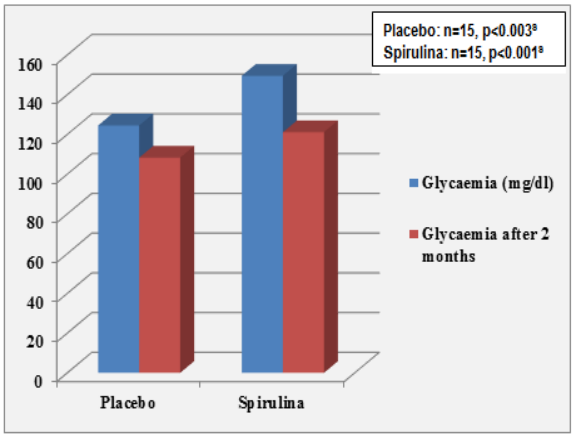

Fig. 1. Evolution of glycemia before and after 2 months compared between groups

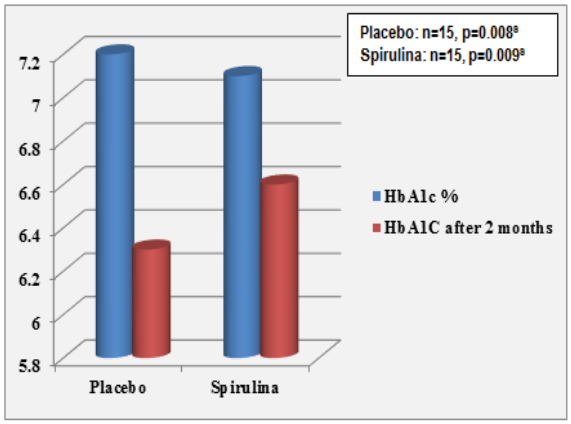

Fig. 2. The evolution of glycated hemoglobin before and after two months compared between groups

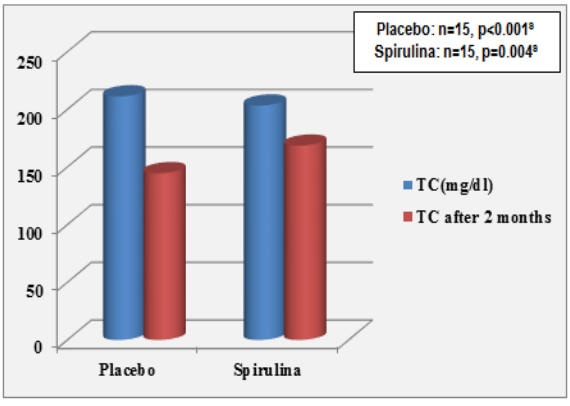

Fig. 3. Evolution of total cholesterol before and after two compared between groups

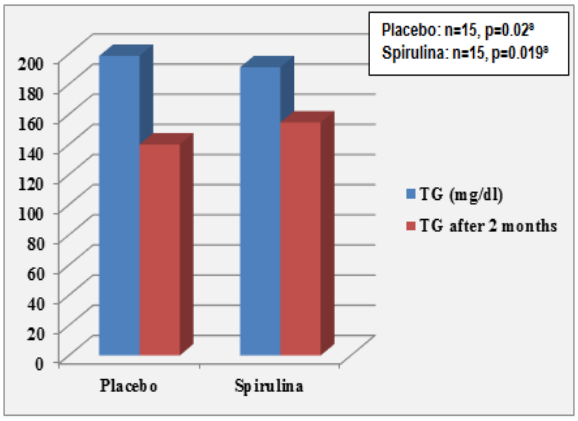

Fig. 4. The evolution of TG before and after two months compared between groups

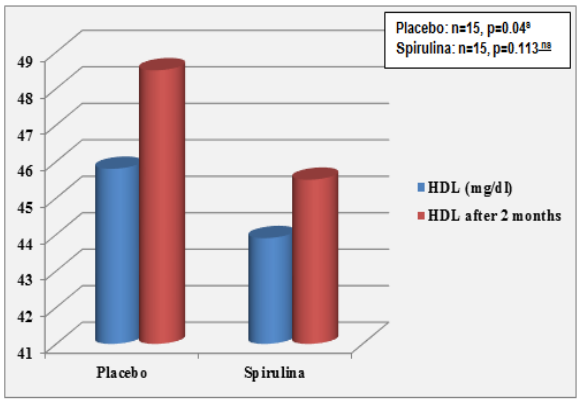

Fig. 5. The evolution of HDL before and after two months compared between groups

DISCUSSION

Our study showed that the supplementation with *Spirulina platensis* tablets has beneficial effects in controlling blood glucose levels and improving the lipid profile of patients with type 2 DM. The lowering effects of Spirulina on blood lipids are not completely known. Spirulina could act on serum lipids by a similar mechanism as statins, inhibiting the hydroxmethylglutaryl coenzyme A reductase and consecutive reduction of cholesterol synthesis [10]. Although the exact biochemical mechanism by which Spirulina reduces lipid levels is not well understood, some studies have presumed that a high C-phycocyanin content might inhibit the activity of pancreatic lipase activity [7]. The content rich in C-phycocyanin is presumed to act together with glycolipid hemoglobin (Hb)-2 to decrease in jejunal cholesterol absorption and ileal bile acid reabsorption [12]. The results showed that spirulina is probably functioning like an insulin-like protein or may stimulate type beta cells of Langerhans to increase the output of insulin, stimulating the decrease of serum glucose levels. Spirulina has an effect which leads to the decrease in level of lipoproteins rich in triacylglycerols. In addition, spirulina could act by a similar mechanism as resins, where reabsorption of bile acids in intestine, and cholesterol conversion to bile acids is inhibited [13]. By an unspecified mechanism, spirulina could affect liver cells through which it intervenes directly in cholesterol formation and thus influences its level in serum. Furthermore, spirulina could affect the metabolism of fatty acids, reduce synthesis of VLDL in the liver and as a result minimize the formation of LDL-C in the circulation. Through antioxidative effect, spirulina has shown to reduce lipoperoxidation in liver [14].

Many studies have shown that during diabetes, excess of glucose present in blood reacts with haemoglobin to form HbA1c [15]. The level of HbA1c is always marked as a reliable index of glycaemic control in diabetes. The reason there is an increase in the level of HbA1c is due to decreased level haemoglobin and increased level of blood glucose. The patients who were given spirulina showed a decrease in level of HbA1c, the results in the spirulina group showed an average value of 7.1± 0.92% before use of spirulina and 6.6 ±0.33% after, (p=0.009), the value of p showed a significant difference in HbA1c. The reason for this could be explained by the fact that spirulina is rich in iron and this contributes to the elevated levels of haemoglobin.

Spirulina platensis is considered a safe product, only few patients reported diarrhea after consumption, being Generally Recognized as Safe (GRAS) compound. Our study, in accordance with previous experimental and clinical trials have shown promising data in the control of type 2 DM and therefore it is expected that spirulina, which acts like an insulin like protein can also be used in the future as an anti-diabetic natural substance, eventually replacing oral medication or even insulin.

CONCLUSION

The results obtained in this study showed a significant reduction of weight, glycemia, total cholesterol and triglycerides values after administration of *Spirulina platensis* supplements at dose of 800 mg daily for two months in patients with type 2 DM. These results reveal that *Spirulina platensis* might be a promising dietary supplement for type 2 DM management. Future well-designed larger trials are required to identify the active ingredients beside Phycocyanin and establish the safety profile of *Spirulina platensis* in humans.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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ROLUL *SPIRULINA PLATENSIS* ÎN CONTROLUL DIABETULUI ZAHARAT TIP 2

REZUMAT
În ultimii ani, numeroase studii experimentale și clinice au evaluat alga albastră *Spirulina platensis* pentru tratamentul diabetului zaharat (DZ). Scopul acestui studiu a fost evaluarea efectelor suplimentelor de Spirulină în controlul DZ de tip 2. Studiul a inclus pacienți cu vârste de 30-70 ani, cu DZ tip 2 diagnosticat de cel puțin doi ani, care au fost tratați cu o doză stabilă de Metformin pentru cel puțin 3 luni. Acest studiu randomizat, singur orb pe grupuri paralele a inclus 30 de pacienți cu DZ tip 2, 15 de pacienți tratați cu Metformin şi placebo (grupul 1) și 15 pacienți tratați cu Metformin și Spirulină (grupul 2). Spirulina a fost administrată de două ori pe zi timp de 2 luni, într-o doză totală de 800 mg pe zi. Următorii parametri au fost evaluați: tensiunea arterială, colesterolul total (TC), trigliceridele (TG), LDL-colesterolul, HDL-colesterolul, valorile serice ale glicemiei și hemoglobinei glicozilate (HbA1c). După 2 luni, s-a observat că valoarea medie a glicemiei a scăzut semnificativ de la 124,5 ± 29,28 mg/dl la 108,3 ± 12,53 mg/dl (p <0,003) în grupul 1 și de la 149,5 ± 19,89 mg/dl la 121,1 ± 10,66 mg/dl în grupul 2 (p <0,001). Valoarea medie a HbA1c scăzut semnificativ de la 7,2 ± 0,63% la 6,3 ± 0,69% în grupul 1 (p = 0,008) și de la 7,1 ± 0,92% până la 6,6 ± 0,33 % în grupul 2 (p = 0,009).
Cuvinte cheie: *Spirulina platensis*, lipide, diabet zaharat tip 2

EFFECTS OF RESVERATROL AND COENZYME Q10 SUPPLEMENTATION IN METABOLIC SYNDROME

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ABSTRACT
Resveratrol and coenzyme Q10 are two nutraceuticals intensively studied for their benefits in several diseases. Their lipid-lowering, anti-inflammatory and antioxidant activities could improve the condition of patients with metabolic syndrome. Our study aimed to evaluate the effects of the association of a dietary supplement containing resveratrol and coenzyme Q10 to the prescribed medication in patients with metabolic syndrome and statin therapy. The study included 29 patients randomized in two groups, one receiving the dietary supplement (n=15) and one receiving placebo (n=14). The serum levels of total cholesterol (TC), triglycerides (TG), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), transaminases and high sensitivity C-reactive protein (hsCRP) have been evaluated at the beginning of the study and after three months. A decrease of serum levels of TC, TG, LDL-C and hsCRP and an increase of HDL-C were noticed. The greater increase of HDL-C in the group receiving the dietary supplement (from 43.80±13.18 to 46.67±10.40 mg/dl) almost reached a statistical significance (p=0.057). The results indicate that the association of resveratrol and coenzyme Q10 in patients with metabolic syndrome could moderately decrease TC, LDL-C, TG and significantly increase HDL-C. Further studies are needed to elucidate the effects of resveratrol combined with coenzyme Q10 on lipid profile parameters.
Key words: metabolic syndrome, resveratrol, coenzyme Q10

INTRODUCTION

Nowadays, metabolic syndrome (MS) is a condition with an increased prevalence, determining a high risk for cardiovascular disease and type 2 diabetes mellitus (DM). Many factors such as the excess calorie diet, the sedentary life habits, and genetic factors could contribute to the development of this disease [1,2]. Several definitions have been proposed for this complex disease by different organizations. The criteria proposed in these definitions for the diagnosis of MS present similarities, but insulin resistance, lipid and blood pressure (BP) levels are present in most of them [3,4]. The central obesity, dyslipidemia, high BP, proinflammatory and prothrombotic state and insulin resistance are the main factors responsible for the evolution to cardiovascular disease (CVD) and type 2 DM [5]. The chronic low-grade inflammation state in metabolic syndrome might be caused by the generation of leptin and adiponectin and different others adipokines by the adipose tissue [6,7].
The main approach in controlling the risk factors of MS is represented by lifestyle changes (increased physical activity, anti-atherogenic diet) [1]. When lifestyle changes are insufficient, the drug intervention combines lipid-lowering, hypotensive and hypoglycemic drugs in MS patients [8]. The polymedication in the same patient increases costs, decreases the compliance and is responsible for many side effects [5]. One of the commonly prescribed classes of drugs to control dyslipidemia in MS patients is represented by statins, leading to a reduction of cardiovascular events [9]. Many side effects like muscle and liver toxicity [10], increase in the incidence of type 2 DM, cognitive impairment have been observed in statin users [11]. It has been shown that statins inhibit cholesterol biosynthesis through decreasing the synthesis of mevalonate and consecutive reduction of coenzyme Q10 levels in the organism [12]. Therefore, an association of statins with coenzyme Q10 supplements might be a good strategy in statin users to improve metabolic parameters [13].
Resveratrol (3, 4', 5 trihydroxystilbene) is a polyphenolic compound that has attracted great interest because of its presence in red wine and its multiple benefits [14-16]. The antioxidant [17], cardioprotective [18], anticancer [19], anti-inflammatory [20], lipid-lowering, anti-hyperglycemic [21] or neuroprotective [22] effects of resveratrol have

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been explored in many experimental and clinical trials. However, the benefits of resveratrol supplementation are still controversial, some recent studies indicating that resveratrol is overestimated [23].
The aim of our study was to evaluate the effect on lipid and inflammation parameters of a dietary supplement containing both resveratrol and coenzyme Q 10 in MS patients on statin therapy.

MATERIAL AND METHOD

This study was randomized, single-blinded with parallel groups and included 29 patients with MS on statin therapy for at least 3 months. The exclusion criteria from this study were represented by any type of cancer or degenerative disease, moderate to severe renal or hepatic disease, acute or chronic inflammatory disease, pregnancy or breastfeeding, abnormal pulmonary X-ray, abnormal ecography, abnormal ECG, deterioration of hematological, hepatic or renal function, hypersensitivity or allergy to any of the compounds of the product administrated in this study, the use of anti-oxidative and anti-inflammatory substances less than two weeks before the start of the study.
The patients enrolled have been distributed in two groups. During three months, the first group (15 patients) received Procor Forte, 1 capsule/day and the second group (14 patients) received placebo. Procor Forte is a dietary supplement containing coenzyme Q10 (60 mg), resveratrol (15 mg), vitamin B6 (3 mg) and magnesium oxide (250 mg) providing 150 g magnesium. For this interventional clinical trial, Sun Wave Pharma Company provided the dietary supplement and the Favisan Company provided the placebo capsules.
The patients have been diagnosed with MS according to the criteria of NCEP-ATP III, presenting 3 or more of the following: waist circumference > 102 cm (men) or > 88 cm (women), triglycerides ≥150mg/dl, HDL cholesterol < 40mg/dl (men) or < 50mg/dl (women), blood pressure ≥ 130/85 mmHg, fasting glucose ≥ 110 mg/dl [8]. Patients had increased cardiovascular risk factors (risk SCORE ≥5). The prescribed medication including statin therapy was respected during the intervention period. Before the beginning of the study patients respected a wash-out period of two weeks in order to eliminate the effects of other dietary supplements or plant extracts consumed before.
At the beginning of the study and after three months the following parameters have been evaluated: anthropometric parameters: weight, height, waist circumference, body mass index (BMI). The serum levels of total cholesterol, triglycerides, LDL, HDL, transaminases, blood glucose, hsCRP and blood pressure were determined in all patients. Bayer diagnostic kit was used to determine the levels of biochemical parameters.
This study was conducted in accordance with the Good Clinical Practice of the European Community, CPMP/ICH/135/95 and the ICH Guidelines for Good Clinical Practice: Consolidated Guidance (GCP E-6, April 1996) / and the Declaration of Helsinki and the applicable local laws and regulations. Written consent was obtained from the patients included in the study. The study was approved by the Ethics Committee of the Institute of Cardiovascular Diseases.
Statistical analysis was done using with the programme SPSS-13, a software package used for statistical analysis by comparison of data from two samples, paired t test was used and unpaired t-test was used respectively when appropriate (1 and 2 tail tests).

RESULTS

The MS patients on statin therapy included in the study presented similar baseline characteristics regarding age, sex, weight, height, BMI. Of the 29 patients 14 were men and 15 women, 6 male and 9 female in the Procor group and 8 male and 6 female in the placebo group. No significant differences have been noted for age (p = 0.289), weight (p = 0.268), height (p = 0.982), BMI (p = 0.222), waist circumference (p = 0.902) as shown in Table I.

Table I. The anthropometric parameters at inclusion

Parameter	Group	Mean ± standard Deviation	p
Age (years)	Procor	57.20±9.29	0.289
	Placebo	61.57±8.90	
Weight (kg)	Procor	96.56±14.23	0.268
	Placebo	100.71±22.04	
Height (cm)	Procor	167.67±8.32	0.982
	Placebo	167.00±5.82	
BMI	Procor	33.53±3.72	0.222
	Placebo	36.22±6.93	
Waist circumference (cm)	Procor	113.40±9.42	0.902
	Placebo	113.14±8.39	

The majority of the patients included in the study received atorvastatin (10 patients in the Procor group and four patients in the placebo group), followed by rosuvastatin (7 patients in the placebo group and 4 in the Procor group). Five patients included in the studied presented side effects to statins (hepatic cytolysis, artralgia and myalgia).
The values of the evaluated parameters in both groups are presented in Table II. For the Procor group (n = 15) significant improvements have been observed for TC with a decrease from 230.40 ± 56.11 mg/dl to 186.13 ± 31.86 mg/dl, LDL-C from 137 ± 38.68 to 114.13 ± 23.39 mg/dl, TG from 167.53 ± 75.99 to 127.07 ± 38.29 mg/dl, ALT from 51.47 ± 50.07 to 18.55±10.33 and hsCRP from 3.73

± 2.19 to 2.67 ± 1.68 mg/dl. No significant improvement was observed for AST levels. The serum values of HDL-C increased from 43.80 ± 13.18 mg/dl to 46.67 ± 10.40 mg/dl (p = 0.057) in Procor group (Table II).

Table II. The parameters in the two groups at inclusion and after 3 months

Parameter		Procor group Mean±Std.deviation	p	Placebo group Mean±Std.deviation	p
Total cholesterol (mg/dl)	Inclusion	230.40±56.11	0.001	233.93±80.89	0.0002
	3 months	186.13±31.86		182.14±47.25	
HDL-C (mg/dl)	Inclusion	43.80±13.18	0.057	45.93±15.97	0.785
	3 months	46.67±10.40		45.57±13.96	
LDL-C (mg/dl)	Inclusion	137.00±38.68	0.003	144.50±54.40	0.018
	3 months	114.13±23.39		119.86±38.36	
TG (mg/dl)	Inclusion	167.53±75.99	0.003	207.21±81.73	0.0002
	3 months	127.07±38.29		149.43±48.39	
ALT (U/L)	Inclusion	51.47±50.07	0.023	36.07±19.84	0.009
	3 months	18.55±10.33		22.79±8.47	
AST (U/L)	Inclusion	36.27±32.30	0.170	33.57±19.63	0.072
	3 months	24.13±6.92		24.43±6.15	
hsCRP (mg/L)	Inclusion	3.73±2.19	0.002	3.43±1.70	0.001
	3 months	2.67±1.68		2.57±1.16	

HDL: high density lipoprotein; LDL: low density lipoprotein; TG: triglyceride; ALT: alanine aminotransferase, AST: aspartate aminotransferase, hs CRP: high sensitivity C-reactive protein

In the placebo group (*n*=14), the serum levels of lipid parameters, transaminases and hsCRP decreased after 3 months of supplementation with placebo: TC decreased from 233.93 ± 80.89 to 182.14 ± 47.25 mg/dl, LDL-C from 144.40 ± 54.40 to 119.86 ±38.36 mg/dl and TG from 207.21 ± 81.73 to 149 ± 48,39 mg/dl, ALT from 36.07 ± 19.84 to 22.79 ± 8.47 and hsCRP from 3.43 ± 1.7 to 2.57 ± 1.16 mg/L. A decrease of serum levels of HDL-C, from 45.93±15.97 mg/dl to 45.57±13.96 has been observed.

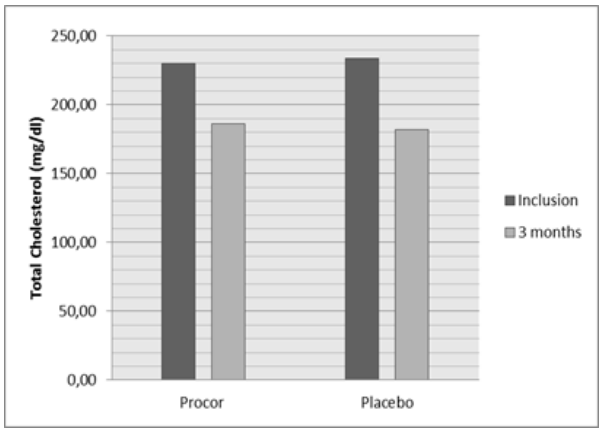

Fig.1. Evolution of total cholesterol levels in Procor and placebo group

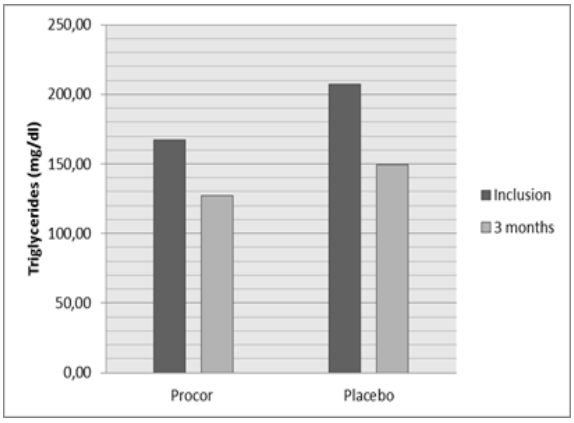

Fig.2. Evolution of TG levels in Procor and placebo group

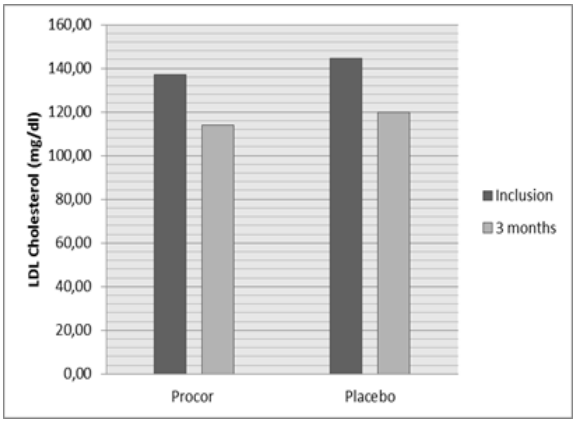

Fig.3. Evolution of LDL-C levels in Procor and placebo group

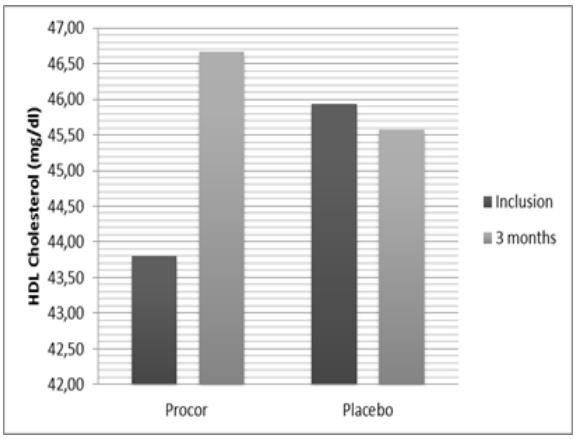

Fig.4. Evolution of HDL-C levels in Procor and placebo group

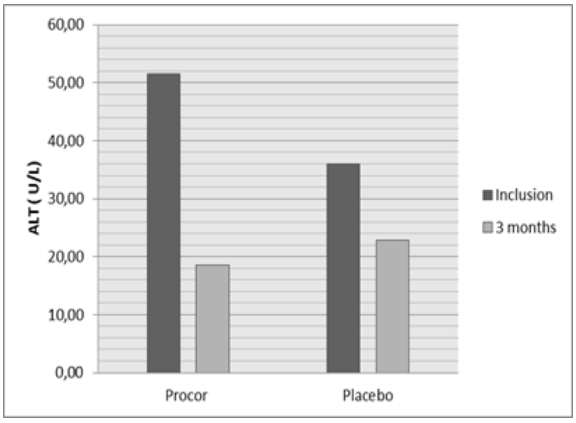

Fig.5. Evolution of ALT levels in Procor and placebo group

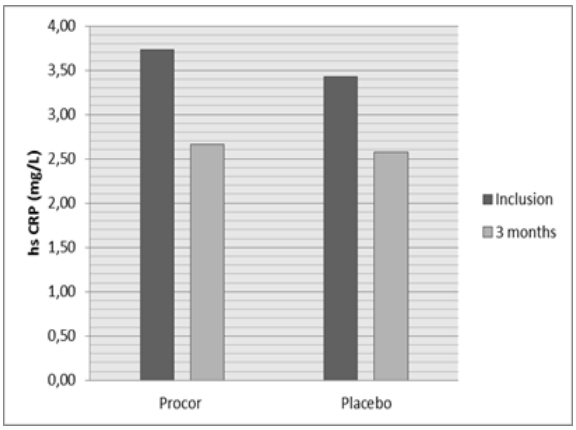

Fig.6. Evolution of hs CRP levels in Procor and placebo group

DISCUSSION

Our study showed positive effects following the administration of a dietary supplement (Procor Forte) containing resveratrol and coenzyme Q10 in patients with MS on statin therapy. The dietary supplement determined an improvement of lipid profile, decreasing TC, LDL-C, and TG and increasing HDL-C (Figures 1-4). The modifications on transaminases and inflammatory parameters like hsCRP were similar in both groups, after Procor or placebo supplementation during three months (Figures 5, 6). The lipid-lowering effects of resveratrol have been noticed in many experimental studies [24,25]. Numerous clinical studies have been performed for resveratrol and coenzyme Q10 to establish the potential benefit of these compounds for health. Even though results indicated by these studies are sometimes controversial, positive effects have been noticed in patients with hypercholesterolemia and type 2 DM [18,26]. A recent study has focused on resveratrol benefits as a dietary supplement in patients with type 2 DM and hypercholesterolemia on statin treatment. The results revealed a decrease of LDL-C, apolipoprotein B and oxidized LDL after six months of administration with a grape supplement enriched in resveratrol [27]. A decrease of serum levels of LDL-C was also noted after the administration of 10 mg of resveratrol daily in patients with coronary artery disease [18].

Our results are in accordance with previous studies on diabetic patients, which point out an increase of serum levels of HDL-C after supplementation with resveratrol (1g/day) [21]. Moreover, the association of coenzyme Q10 with statin therapy might also increase the serum levels of HDL-C. A study evaluated the benefits of the association of coenzyme Q10 and statins, and revealed increased serum levels of HDL-C in patients on rosuvastatin, compared to patients on atorvastatin [28]. Atorvastatin seems to determine a more evident decrease of ubiquinol levels than rosuvastatin does [28]. Our results indicated an increase of serum levels of HDL-C in the Procor group, supporting the evidence that the association of coenzyme Q10 to statin can improve serum lipid parameters.

The dose of resveratrol present in the dietary supplement was 10 mg. Although other clinical trials have used higher doses of resveratrol, benefits have been observed even al lower doses. Brasnyó et al used in their study capsules containing 5 mg of resveratrol (twice daily) and observed an improvement in insulin sensitivity [29].

The small number of patients included in the study and the short period of intervention could explain the moderate effects of this dietary supplement containing mainly coenzyme Q10 (60 mg) and resveratrol (15 mg) on lipid profile parameters.

CONCLUSION

The association of a dietary supplement containing resveratrol and coenzyme Q10 in patients with metabolic syndrome on statin therapy can improve the lipid profile, mainly through increasing the levels of HDL-C. Further studies conducted on a larger period of time and including an increased number of patients are needed to establish the benefit of the association of resveratrol and coenzyme Q10 in patients wih MS.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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EFECTELE SUPLIMENTELOR ALIMENTARE CONȚINÂND RESVERATROL ȘI COENZIMA Q10 LA PACIENȚII CU SINDROM METABOLIC

REZUMAT

Resveratrolul şi coenzima Q10 sunt două nutraceutice intens studiate pentru beneficiile lor în mai multe boli. Activitățile lor hipolipemiante anti-inflamatorii şi antioxidante ar putea îmbunătăți starea pacienților cu sindrom metabolic. Studiul nostru a urmărit evaluarea efectelor asocierii un supliment alimentar care conține resveratrol și coenzima Q10 la medicamentele prescrise la pacienţii cu sindrom metabolic şi tratament cu statine. Studiul a inclus 29 de pacienţi randomizaţi în două grupuri, unul care a primit suplimentul alimentar (*n* = 15) și un grup care a primit placebo (*n* = 14). Nivelurile serice ale colesterolului total (TC), triglicéridelelor (TG), LDL-colesterolului (LDL-C), HDL-colesterolului (HDL-C), transaminazelor și proteinei C reactive cu sensibilitate crescută (hsCRP) au fost evaluate la începutul studiului și după trei luni. S-a observat o scădere a nivelurilor serice ale TC, TG, LDL-C și hsCRP și o creștere a HDL-C. Creşterea HDL-C observată în grupul care a primit suplimentul alimentar (de la 43.80 ± 13.18 la 46.67 ± 10.40 mg / dl), s-a apropiat de valoarea semnificativă din punct de vedere statistic (*p* = 0,057). Rezultatele prezentului studiu indică faptul că asocierea resveratrolului și coenzimei Q10 la pacienţii cu sindrom metabolic ar putea scădea moderat TC, LDL-C, TG și crește semnificativ valoarea HDL-C. Sunt necesare studii suplimentare pentru a elucida efectele combinaţiei resveratrol şi coenzima Q10 asupra parametrilor profilului lipidic.

Cuvinte cheie: sindrom metabolic, resveratrol, coenzima Q10

NOVEL INSIGHTS INTO THE ROLE OF METHYLENE BLUE IN MITOCHONDRIAL PROTECTION

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ABSTRACT
Mitochondrial dysfunction and reactive oxygen species (ROS) generation are critical events in the pathophysiology of ageing and chronic disorders, in the past years modulation of mitochondrial function becoming thus an important therapeutic target. Pharmacological agents able to improve mitochondrial function and/or to decrease ROS production are nowadays highly investigated. Among them an emerging compound is methylene blue (MB), a tricyclic phenothiazine, used for more than a century to treat a variety of disorders. Due to its redox activity, MB has a high affinity for numerous tissue oxidases, including those within mitochondria. Here we briefly review the use of MB in medicine and biology and discuss its beneficial effects as a mild-redox agent able to prevent mitochondria-driven disorders. Methylene blue has a significant potential to improve mitochondrial metabolism and represents a promising therapeutic approach in several conditions associated with mitochondrial dysfunction.
Key words: mitochondria, methylene blue, redox potential

INTRODUCTION

Historically, methylene Blue (MB), a tetramethylthione hydrochloride ($C_{16}H_{18}ClN_3S$) is a heterocyclic aromatic compound firstly synthesized by Heinrich Caro and used as a cotton staining dye [1]. In the 19th century, Peter Ehrlich demonstrated its beneficial effects in the treatment of malaria [2]; this report represented the started point for investigating the role of MB in the field of bacterial and viral infections [3] and cancer [3,4]**.** MB was further used both as therapeutic (e.g., antiseptic, cancer chemotherapy drug) and diagnostic compound (e.g., staining in neuroanatomy and bacteriology, redox agent in biochemical studies) [4,6].
US Food and Drug Administration (FDA) approved MB in treating methemoglobinemia, either inborn [7] or after an overexposure to drugs, nitrophenols, and environmental poisons (e.g., nitrates or cyanides) [8]. The beneficial effects of MB in methemglobinemia therapy are due to its unique autooxidyzing property: indeed, as stipulated by Rojas et al. (2012), "its reduction-oxidation capacity allows electron cycling, without MB gaining any permanent stoichiometric or net reduction" [9](Figure 1). This property explains how MB is acting as an electron donor in the nonenzymatic reduction of methemoglobin: the NADPH methemoglobin reductase reduces MB to LeucoMB (Figure 1), with a nonenzymatic transfer of electrons to methemoglobin, and by thus restoring the functional hemoglobin and MB [10]. Therefore, same Paul Ehrlich named it the "magic bullet" due to its autooxidyzing property for electron cycling, which was firstly identified in the nervous tissue [11].

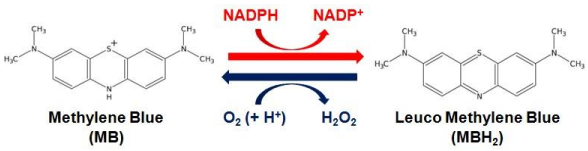

Fig. 1. Methylene blue: a redox active agent. NADPH reduces MB (blue color) to MBH_2 (colorless) which is further oxidized by O_2 (modified after 12).

MB has a high-affinity for the nervous tissue [13], being used firstly in 1938 in the experimental treatment of schizophrenia [14]. Since then, MB has proved to be a neuroprotective agent in numerous neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), Leber's optic neuropathy [7, 15-22]. The sulfur atom within the structure of MB compound has been suggested to be responsible for this high-affinity to neural structures, yet the underlying mechanism is not completely understood [23].

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In the past decade, an increasing number of studies investigated the neuroprotective role of MB in various pathological conditions: accordingly, MB reduced the cerebral damage induced by ischemia/reperfusion (I/R) in a model of transient focal cerebral ischemia and attenuated the behavioral and neurochemical impairment in a PD model [24]. Furthermore, MB prevented the decline of the cognitive function in transgenic models of AD and slowed the progression of the disease in a clinical trial [25–27].

MB's protective effects have been also studied in relation with ageing [28] and in pathological states associated with liver failure due to intestinal I/R injury [29]. Thus, MB (at nM levels) was proven to extend the life span of human IMR90 fibroblasts in tissue culture by enhancing mitochondrial function: it increased mitochondrial complex IV, enhanced cellular oxygen consumption, increased heme synthesis, and reversed premature senescence caused by H_2O_2 or cadmium [28]. Collange et al. (2013) demonstrated that the intra-peritoneally injection of MB before reperfusion restored the oxidative capacity of liver mitochondria after I/R injury of the gut, a result which could have beneficial clinical implications during vascular surgery by decreasing the incidence of postoperative multiple organ dysfunction [29].

Nowadays, MB is clinically used in multiple disorders and diagnostic procedures (see Table I).

Table I. Clinical indications of MB and corresponding dosage (after 25 and 30)

Clinical indications of MB	MB dosage/Ref.
Inherited methemoglobinemia	1 x 50–250 mg/day (for lifetime) (7)
Acute methemoglobinemia	1–2 x 1.3 mg/kg (i.v. over 20 minutes) (30)
Ifosfamid-induced neurotoxicity	4 x 50 mg/day p.o. or i.v. (18)
Prevention of urinary tract infections in elderly patients	3 x 65 mg/day p.o. (30)
Vasoplegic adrenaline-resistant shock	200 mg i.v. over 1 hour followed by infusion (0.25–2 mg/kg/hour) (31)
Alzheimer's disease	3 x 60 mg/day (Rember™ according to 32)
Pediatric malaria	2 x 12 mg/kg p.o. for 3 days (30)
Parathyroid imaging	3–7.5 mg/kg i.v. (25)
Sentinel lymph node biopsy	Local application of 1–5mL of 1% MB (25)

i.v.: intravenous; p.o., oral.

Methylene Blue and Mitochondrial Function

An important approach to improve mitochondrial function is represented by agents with mild redox activity. It is well known that mitochondria are rich in the redox centers (heme, flavin mononucleotide, flavin adenine dinucleotide, iron-sulfur, copper, or natural coenzyme Q) which represent the major source of free radicals production [33]. Thus, since mitochondria physiology depends on major redox activities, redox active agents have been recently proposed as mitochondrial modulators able to enhance energy production and/or to decrease oxidative stress [33].

The mitochondrial electron transport chain (ETC) at the inner mitochondrial membrane is composed of five multimeric complexes (complexes I to V) and two intermediary substrates (coenzyme Q and cytochrome c). The reduced $NADH+H^+$ and $FADH_2$ produced by the intermediate metabolism are further oxidized by the mitochondrial ETC to induce an electrochemical gradient of protons from the mitochondrial matrix to the inter-membrane space (Figure 2), which is finally used by the F_1F_0-ATP synthase (complex V) to produce ATP [34].

Atamna et al. (2012) suggested that redox agents with a mild redox potential (-0.1 to 0.1 V) are able to improve the mitochondrial function via "bypassing the production of superoxide radicals and the cycling between the reduced and oxidized form": mild redox agents enter the cell in the oxidized form, are reduced by the physiologic redox centers (e.g., NADH-dehydronase of complex I), and are further re-oxidized by a second physiologic center (e.g., cytochrome c) [33]. The redox potential of MB is ~ 10 mV, which is considered mild *vs.* the redox potential of the ETC components [28], thereby MB serves as an electron carrier increasing the mitochondrial energy production and inhibiting the superoxide production [35].

As several other drugs, MB presents hormetic pharmacological effects: at low doses elicits beneficial effects, whereas in high concentrations might become deleterious [9]. To enter cytosol, MB is reduced at the cell surface to its lipophilic form, MBH_2. Inside the cell, MBH_2 is re-oxidized to MB by the heme proteins. At low doses, there is an MB-MBH_2 equilibrium (electron cycling) and MBH_2 can donate electrons to ETC complexes and oxygen, leading to enhanced energy metabolism and decreased superoxide formation. At high doses, this MB-MBH_2 equilibrium is impaired and MB can take electrons away from ETC complexes, leading to decreased activity of these complexes and promoting oxidative stress [9].

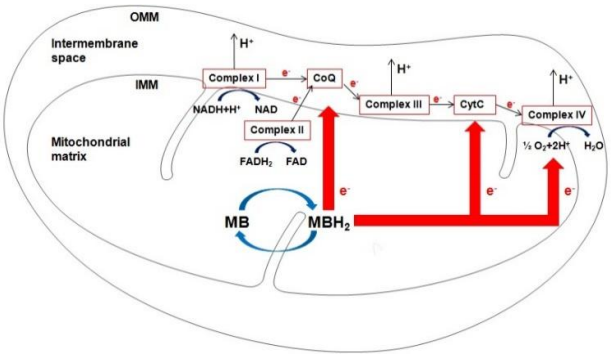

Fig. 2. Schematic representation of methylene blue action within mitochondria (modified after 9). Methylene blue acts as an artificial electron donor to: (i) mitochondria electron transport chain complexes, increasing thus the energy production, and (ii) oxygen, preventing superoxide formation. (IMM - inner mitochondrial membrane; OMM – outer mitochondrial membrane). *Illustration realized thanks to Servier Medical Art.*

MB hormesis is strongly linked to the mitochondrial membrane potential: a high membrane potential will be responsible for an important accumulation of MB and the dimerization of MB molecules [9]. Gabrielli et al. (2004) demonstrated that oxidative radicals are highly produced in the presence of MB monomers and in a lesser extent in the presence of MB dimers [36]. Thus, low concentrations rather than high concentrations of MB, are associated with enhanced mitochondrial metabolism [37]. In line with these observations, we recently investigated the effects of 5 progressive concentrations of MB (0.05, 0.1, 1, 5, and 10 μM, respectively) on oxygen consumption rate (OCR) and the extracellular acidification rate (ECAR), respectively in H9c2 cardiomyoblasts [38]. We clearly demonstrated the hormetic effect of the acute administration of MB: 0.1 μM induced an increase in both OCR and ECAR, indicating an overall stimulatory impact on cellular bioenergetics. However, all OCR linked parameters and ECAR were decreased in a concentration-dependent manner when MB was administered in the higher concentrations (1, 5, and 10 μM), and were not influenced by 0.05 μM MB, respectively [38]. Our results are in agreement with those reported by Atamna et al. (2008) who showed that human IMR90 fibroblasts in tissue culture exposed to high (micromolar range) but not to low (nanomolar range) concentrations of MB showed a compensatory upregulation of antioxidant enzymes with decreased heme expression and iron uptake [28]. Moreover, high concentrations of MB induced the alteration of neural structure or function *in vivo*, including humans [37, 39-43]. Another important finding is that not only acute administration of MB is beneficial; chronic administration of low doses of MB increased complex IV activity in brain and heart of old mice, explaining thus the preservation of mitochondrial function and energy metabolism [9, 28, 44, 45].

CONCLUSIONS

It is widely accepted that preventing mitochondrial dysfunction in both acute and chronic pathologies has significant clinical benefits, an observation that rendered mitochondria in the past decades an attractive therapeutic target. In this regard, when administered in an appropriate doses, methylene blue has a significant potential to improve mitochondrial respiration and decrease superoxide radicals formation, via its mild redox potential. Further studies aimed at dissecting the molecular and cellular mechanisms underlying the beneficial effects of methylene blue are definitely warranted.

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NOI PERSPECTIVE ASUPRA ROLULUI ALBASTRULUI DE METILEN ÎN PROTECȚIA MITOCONDRIALĂ

REZUMAT

Disfuncția mitocondrială și producerea de specii reactive de oxigen (SRO) constituie evenimente critice în fiziopatologia procesului de îmbătrânire precum și a numeroase afecțiuni cronice, astfel că modularea funcției mitocondriale a devenit o importană țintă terapeutică în ultimii ani. În consecință, agenții farmacologici capabili să îmbunătățească funcția mitocondrială și/sau să inducă reducerea producției de SRO sunt acutalmente intens investigați. Printre aceștia, un compus extrem de important este albastrul de metilen (AM), un compus fenotiazinic triciclic, folosit de mai bine de un secol în tratarea unor afecțiuni diverse. Datorită potențialului său de tip redox, AM prezintă o afinitate crescută pentru numeroase oxidaze tisulare, incuzându-le și pe cele de la nivel mitocondrial. Vom prezenta pe scurt utilizarea AM în medicină și biologie, precum și rolul benefic al AM ca și agent de tip redox capabil de a preveni afecțiunile induse de disfuncția mitocondrială. Albastrul de metilen prezintă un potențial semnificativ de îmbunătățire a metabolismului mitocondrial, reprezentând astfel o abordare terapeutică promițătoare în afecțiunile induse de disfuncția mitocondrială.

Cuvinte cheie: mitocondrii, albastru de metilen, potențial redox