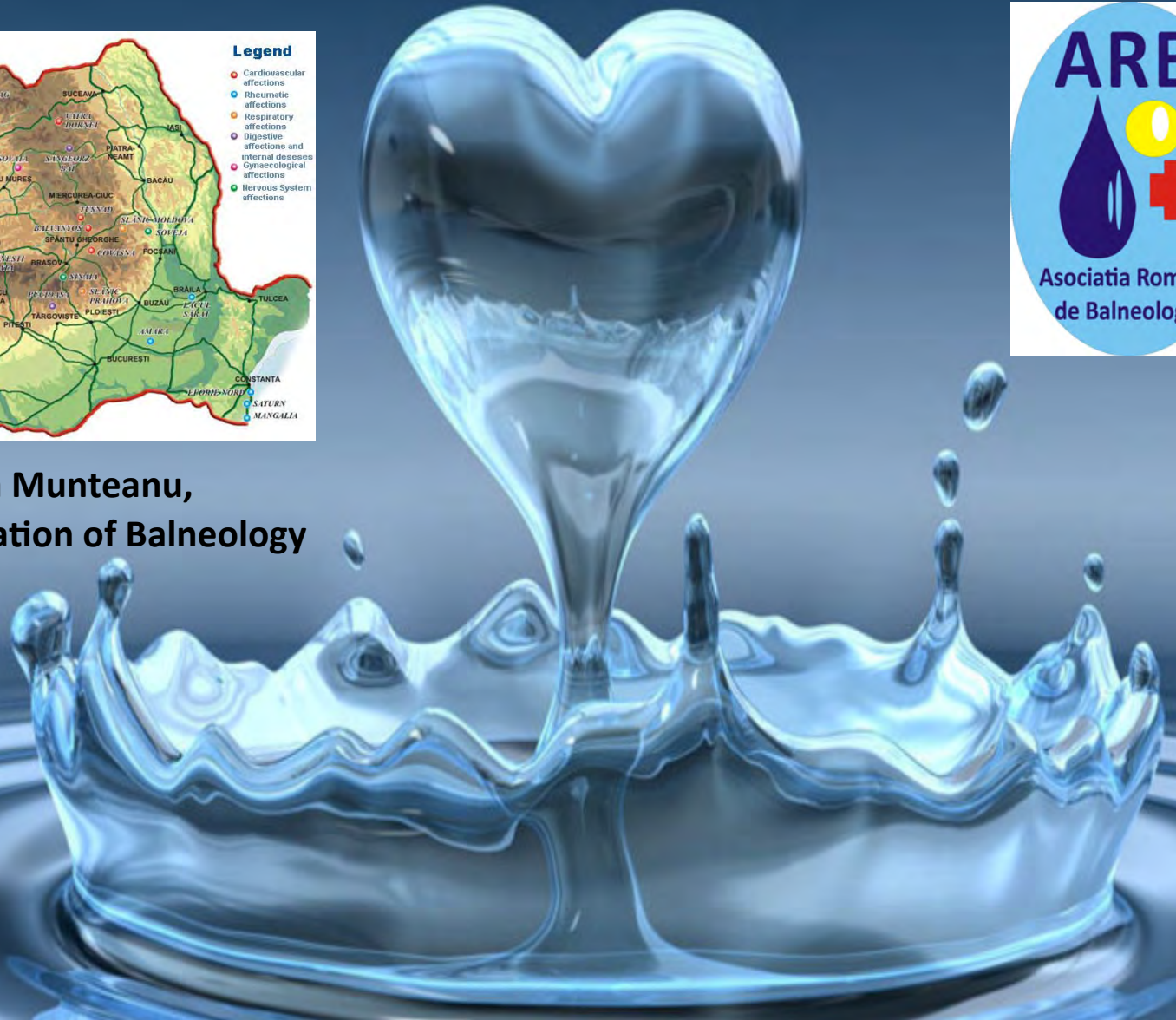


Balneological research in Romania



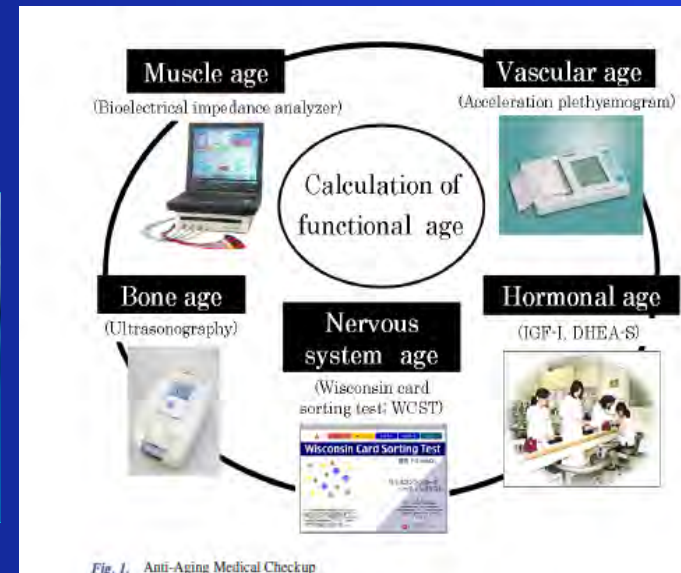
**Constantin Munteanu,
Romanian Association of Balneology**



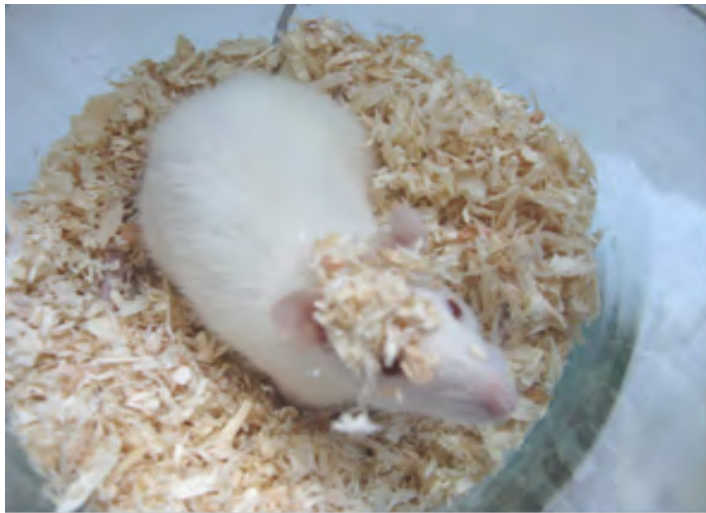
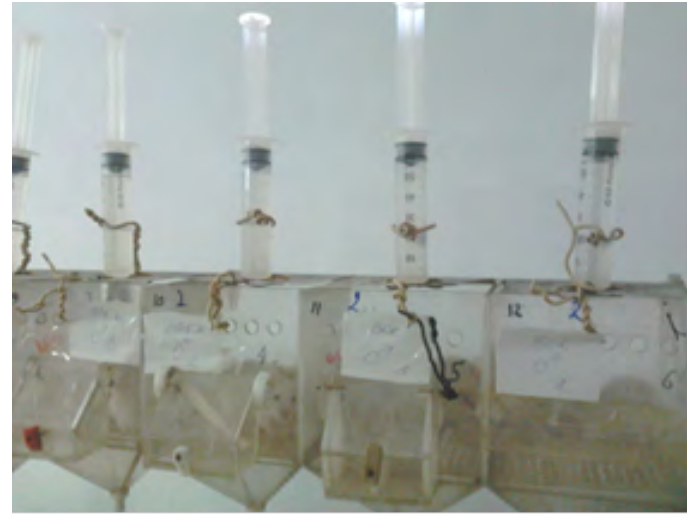


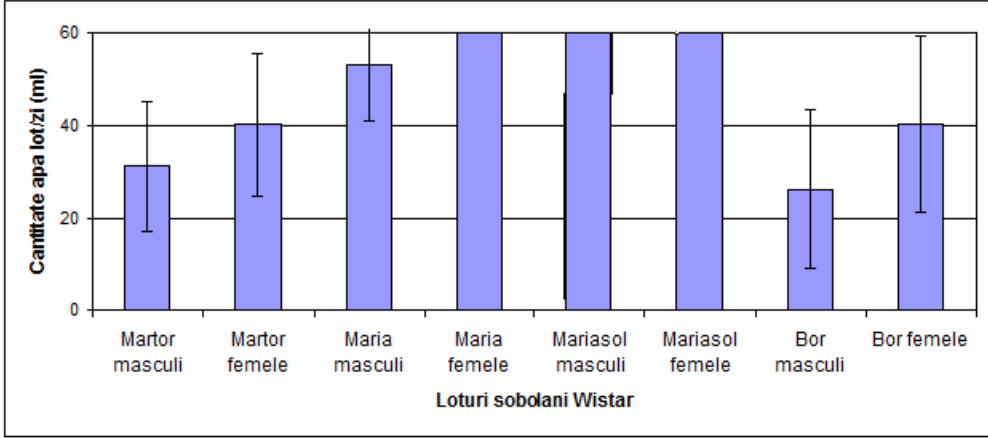
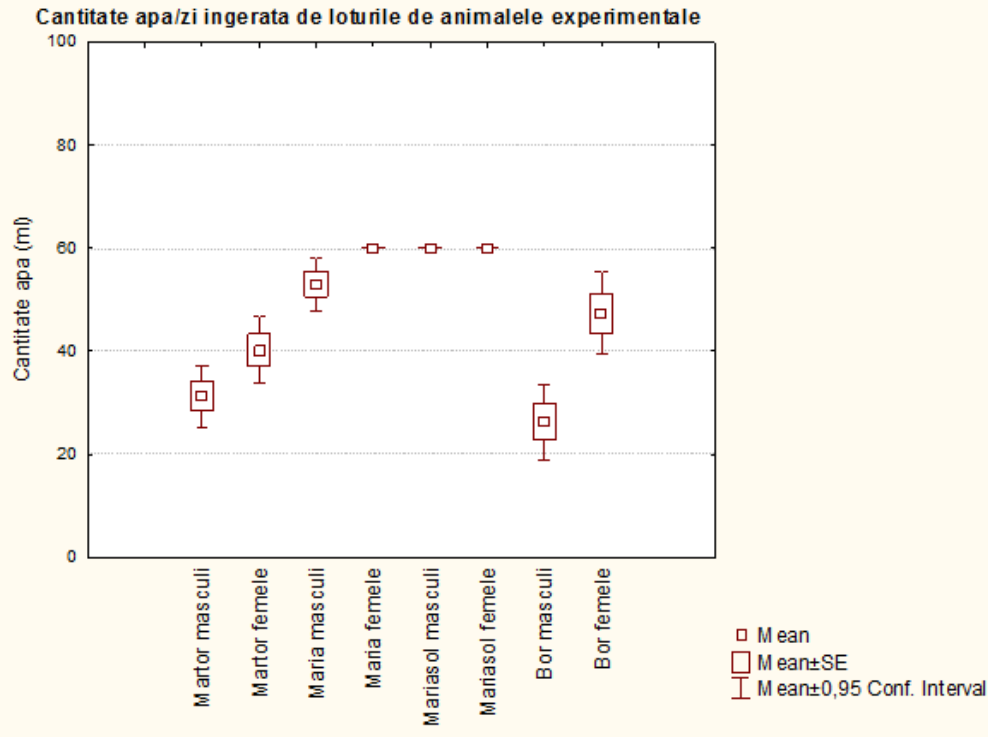
Biomarkers

- In the context of the complex picture of early diagnosis, treatment and prevention of diseases associated with age, picture containing many unquantifiable and independent variables, difficult to analyze, appears to be necessary the analysis, mathematical modeling and simulation of bio-medical relations of laboratory parameters.

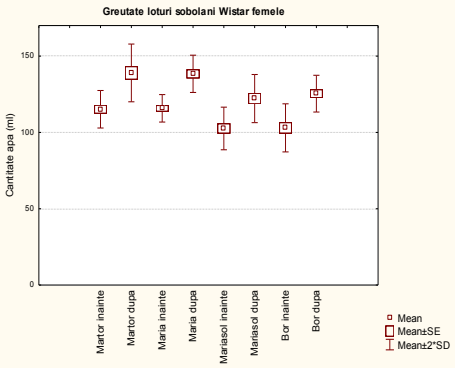
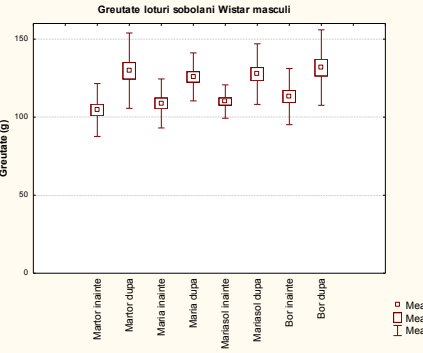


Animal model study: effects of Mary Mineral Water from Malnas Bai, Romania



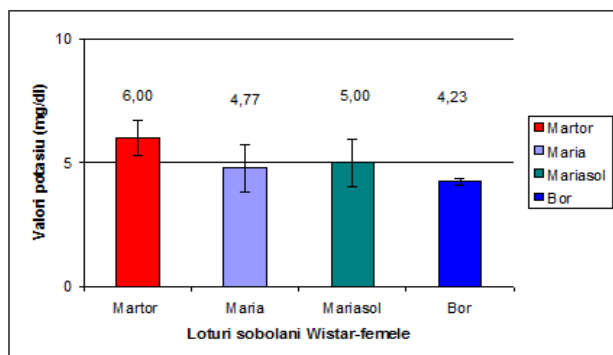
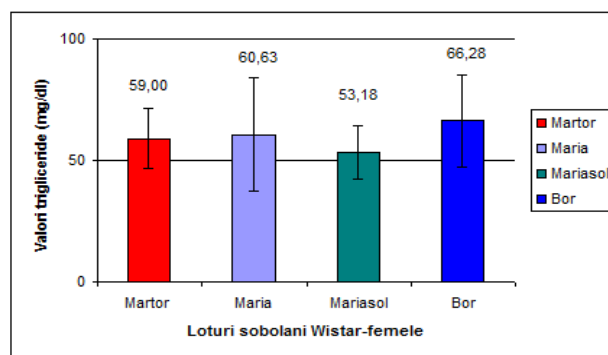
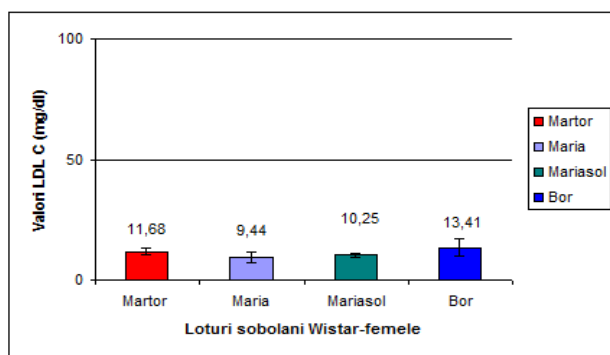
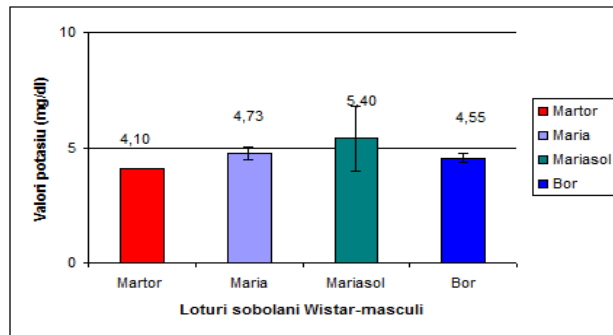
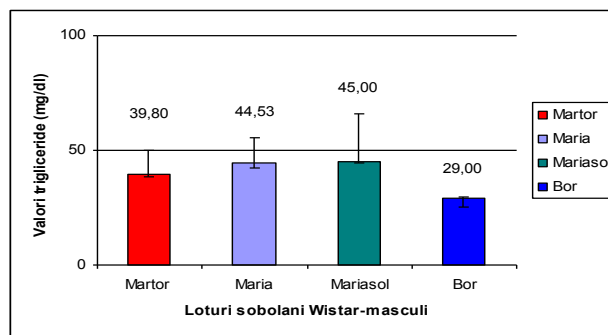
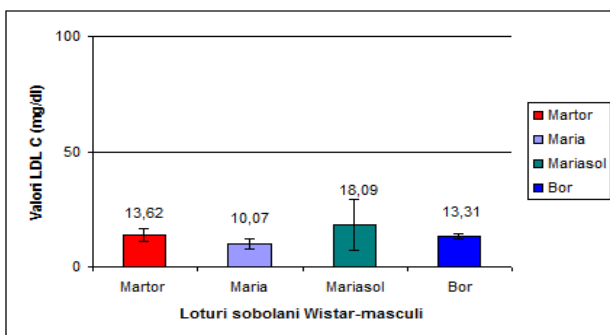
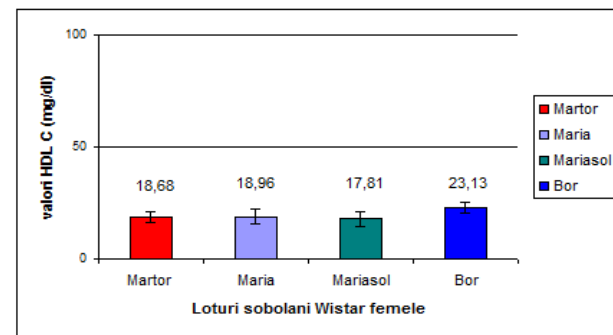
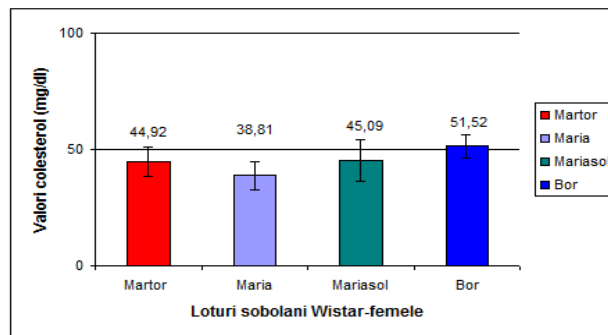
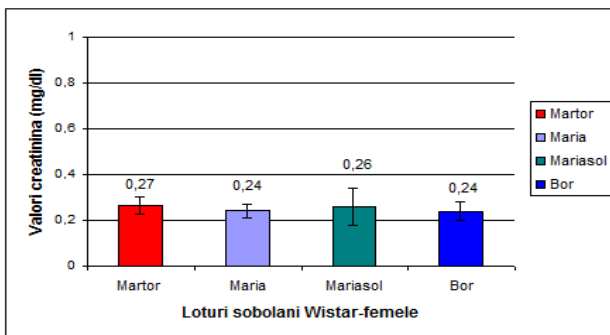
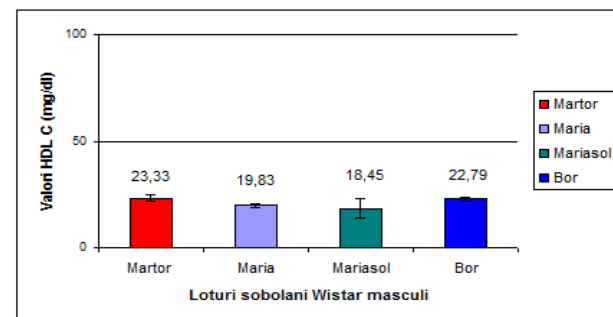
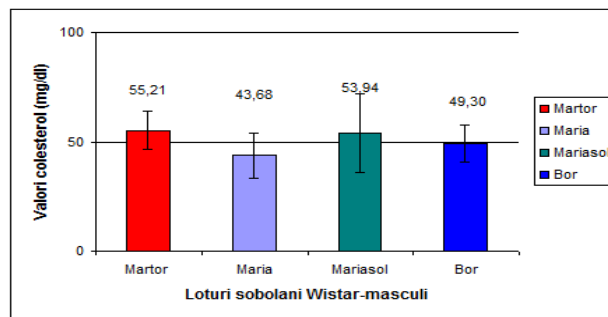
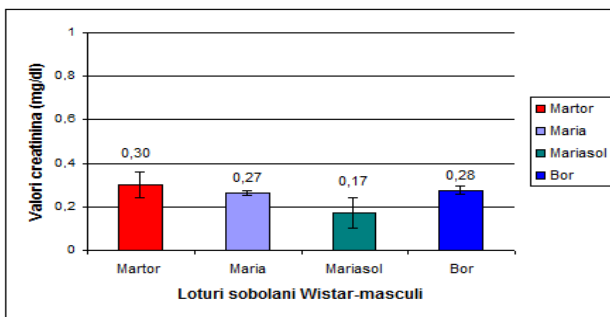


Loturi sobolani masculi	Greutate (g)	
	inainte	dupa
Martor	108	113
	107	125
	106	132
	112	133
	90	146
Maria	111	130
	101	135
	100	128
	116	120
Mariasol	116	116
	113	116
	112	143
	103	126
	116	126
Bor	106	127
	112	116
	118	144
	126	134
	104	123
	106	142



Loturi sobolani femele	Greutate (g)	
	inainte	dupa
Martor	120	128
	108	143
	123	153
	113	135
	112	136
Maria	108	138
	119	143
	118	132
	118	133
	116	146
Mariasol	111	126
	108	124
	94	120
	102	131
	98	110
Bor	98	127
	113	135
	110	120
	96	124
	98	121





CARBOGASEOUS MINERAL WATER FOR PATIENTS WITH METABOLIC SYNDROME

Daniela Poenaru, Delia Cinteza, Constantin Munteanu, Victorita Marcu, Sebastian Diaconescu, Dan Dumitrascu, Horia Lazarescu
National Institute of Rehabilitation, Physical Medicine and Balneology – Bucharest, Romania



HCO₃: 1903,2 mg/l,
CO₂: 2868,4 mg/l
Ca: 384,4 mg/l
Mg: 107, 1 mg/l,
Total mineralization: 2554 mg/l.

- **Introduction**

The carbogaseous mineral water from Borsec, nr 1 spring, was intensively studied before '90; its effect on decreasing the level of glycemia in diabetics are well known. This results were reinforced in more recent studies. Traditionally, this mineral water is also used for people with metabolic and endocrinologic disorders.

- **Objective**

The present study intends to evaluate the influence of carbogaseous mineral water from Borsec, nr 1 spring, on the components of metabolic syndrome

- **Materials & Methods**

The study is a prospective one, single-blind, controlled.

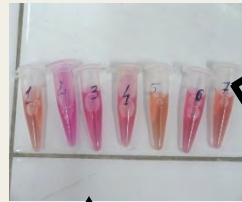
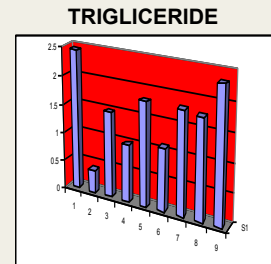
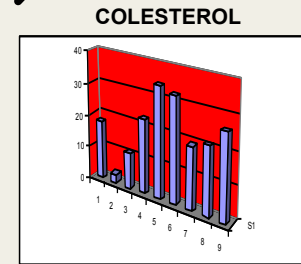
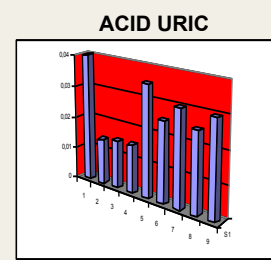
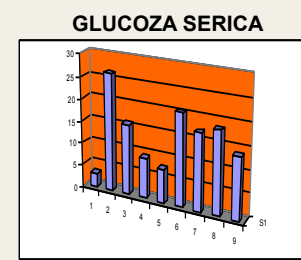
45 patients with metabolic syndrome were divided in 3 groups; group A received tap water, group B (study group)- carbogaseous mineral water r and group C- plain water, for 3 weeks, 2 liters daily.

The following biological parameters were determined at the beginning and after 3 weeks: MCP – 1 (monocyte chemoattractant protein-1), Human MCSF (macrophage colony stimulating factor), TNF beta, Interleukine 6, PCR high sensitivity, Glycemia, Cholesterol (total, LDL, HDL), Triglycerides, Uric acid, Fibrinogen.

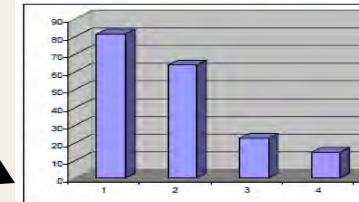
- **Results**

The statistical analysis of the obtained data didn't find significant differences between the groups, but the results are encouraging.

Some of the data showed favorable improvement for the study group of the level of glycemia, uric acid, cholesterol, both HDL and LDL fractions, even these results are not statistical significant



IL6, CRP, MCSF, TNF- β , MCP

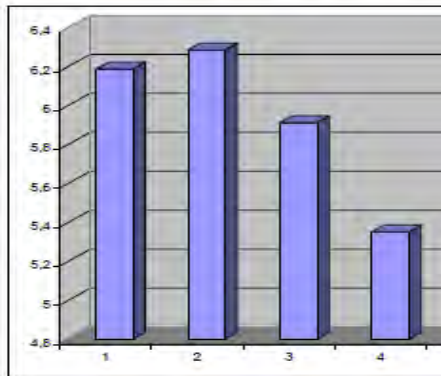


Scientific arguments

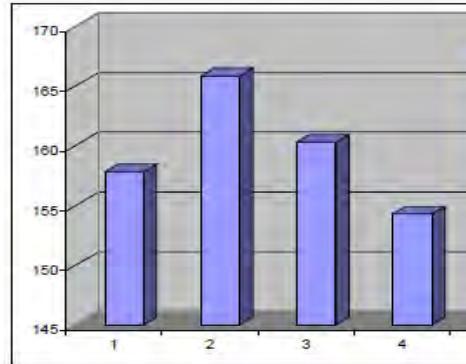
Experimental Design



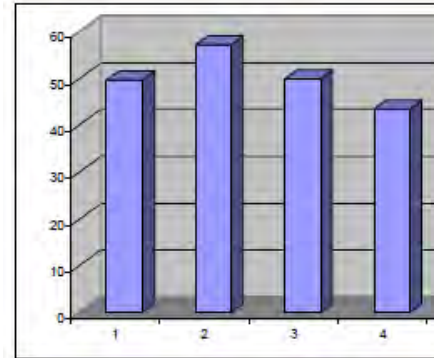
Uric acid



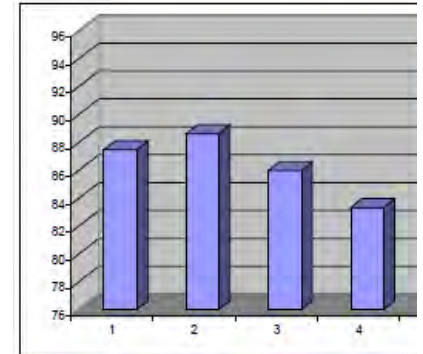
Cholesterol



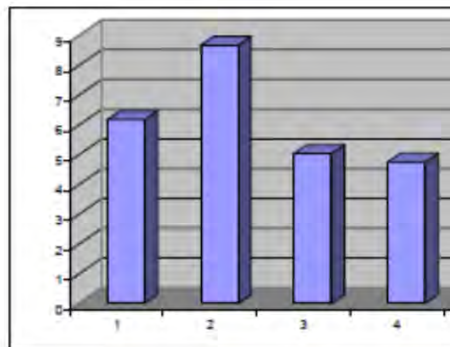
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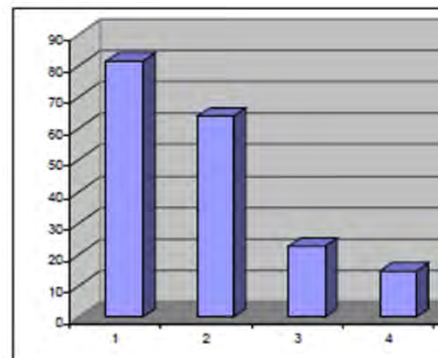
LDL



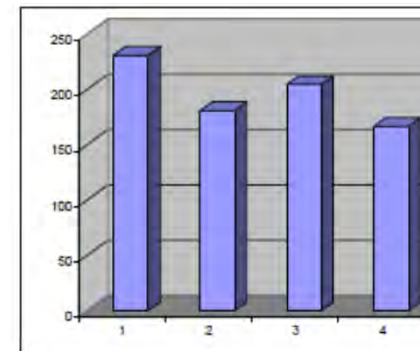
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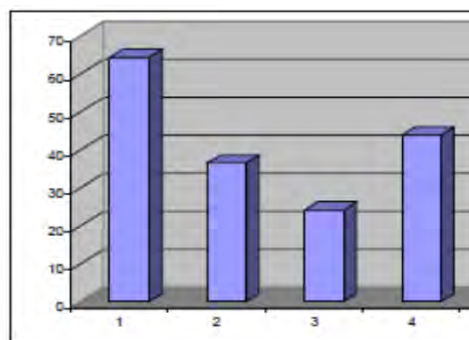
IL6



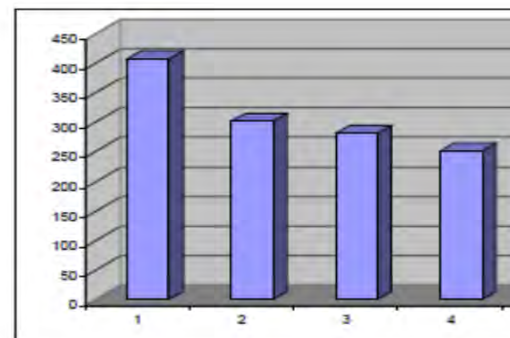
MCP-1



MCSF

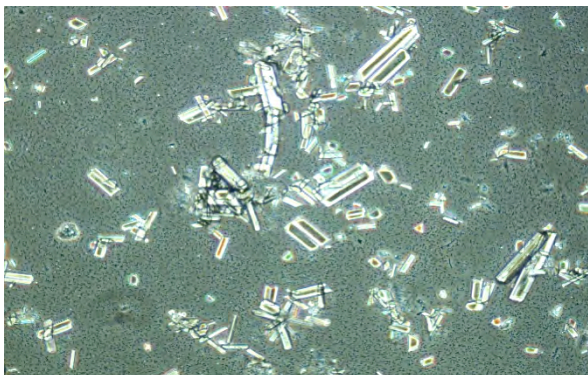
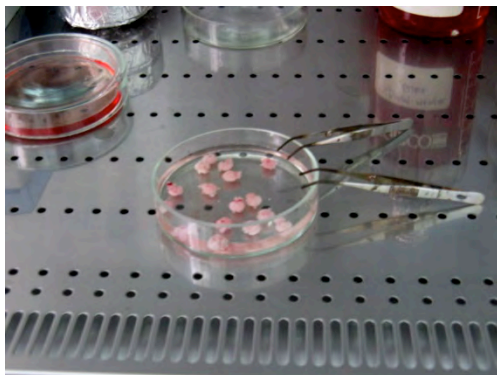
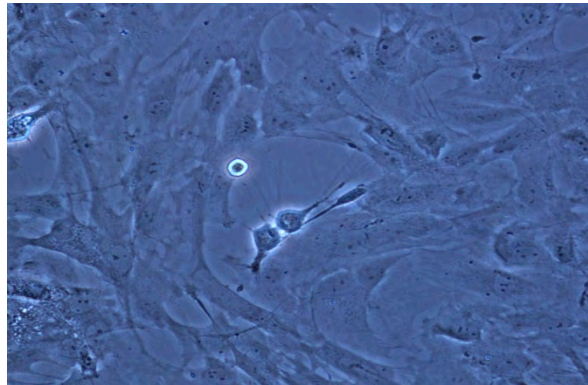
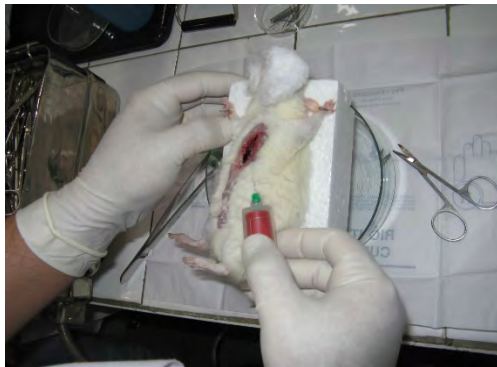
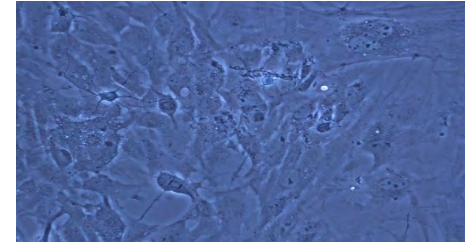


TNF- β

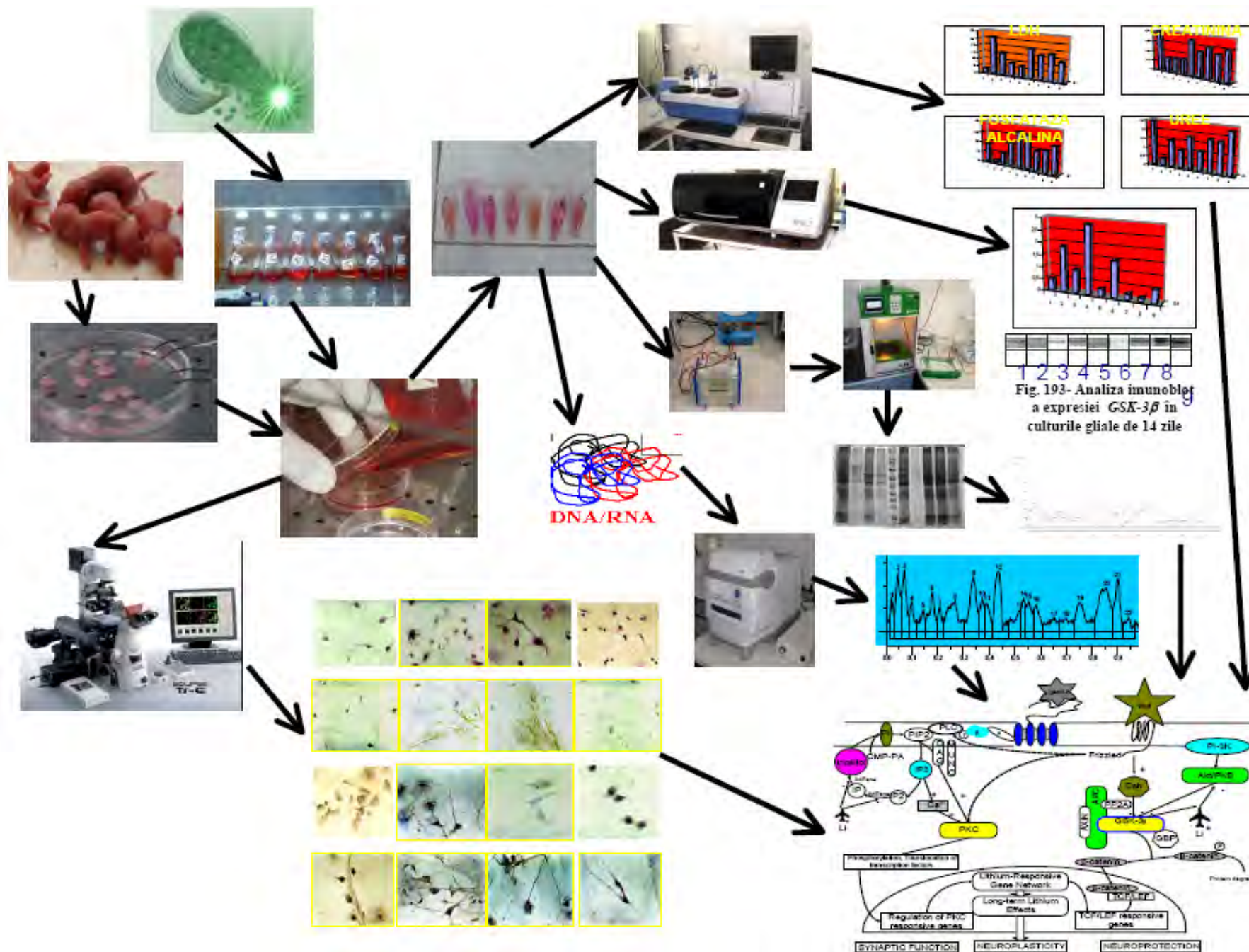


Conclusions: Drinking cure of carbogazeous water of nr. 1 Borsec spring had demonstrate good clinical effects in lowering the serum level of glycemia an uric acid. This study open a gate to show more deep effects on the markers of inflammation. It is possible to have one more weapon in our fight to prevent cardiovascular and cerebrovascular diseases.

ADVANCED RESEARCH AT CELLULAR AND MOLECULAR LEVEL



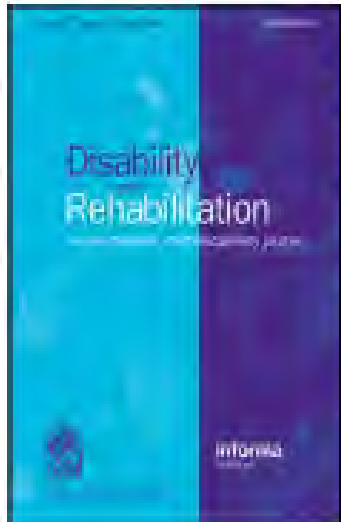
In vitro studies allows evaluation of cell morphology, protein synthesis, secretion of certain substances, cell metabolism, cellular receptors interaction with different ligands, uptake or release of electrolytes or other types of substances that reach the cellular environment.



Speleotherapy: a special kind of climatotherapy, its role in respiratory rehabilitation

Tibor Hervath*

* Municipal Hospital, Tapolca, Hungary



Speleotherapy, the use of the climate of caves, is an accepted but not widely known therapeutic measure in the treatment of chronic obstructive airway diseases. This study summarizes the therapeutic experience of more than 4000 patients who were treated in a 10-year period in a hospital-cave complex in Tapolca, Hungary. A sharp and long-lasting clinical improvement and a significant recovery from airway obstruction could be observed in the overwhelming majority of patients. It is established that the microclimate of some caves can beneficially affect these disorders and the cave should be considered as an optimal environment for complex respiratory rehabilitation.



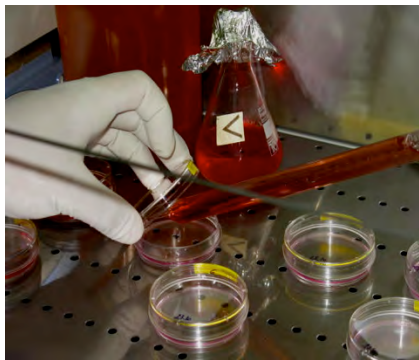
Objective: To explore the effects of speleotherapy on cellular morphology and physiology of pulmonary fibroblasts obtained from tissues of Wistar rats, in normal and Ovalbumin challenged “asthmatic” conditions.

Materials and methods:

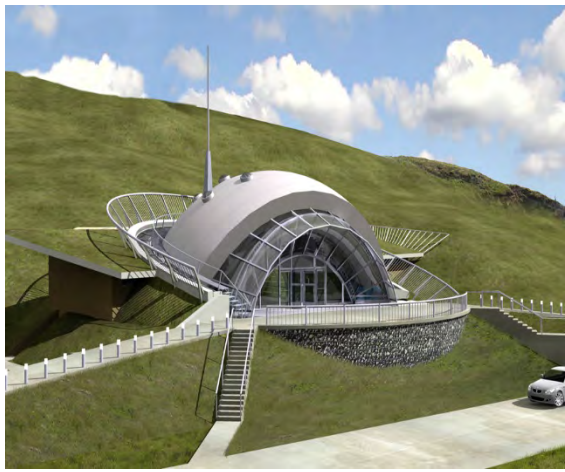
Wistar rats of 75-100 g weight were divided in two lots: control and ovalbumin challenged animals. Ten animals of each lot were send to Turda, Cacica and Dej Salt Mine for 14 days and maintained in the salt mine medium, as in speleotherapy treatment.



Pulmonary fibroblasts cultures were prepared from Wistar rat lung Assessing changes in cellular and molecular level can be achieved by optical microscopy, immuno-histo-chemistry studies, electrophoresis and Western blotting. The proteins electrophoresis from the total homogenate has as the purpose to establish the changes, which are revealed at the proteic level of fibroblasts cultures obtained from rats held on saline mine medium for speleotherapy.

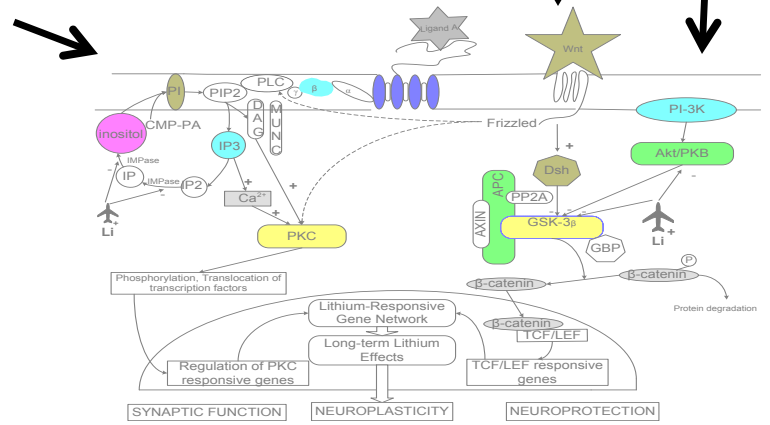
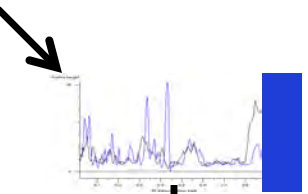
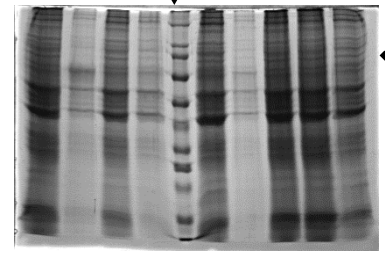
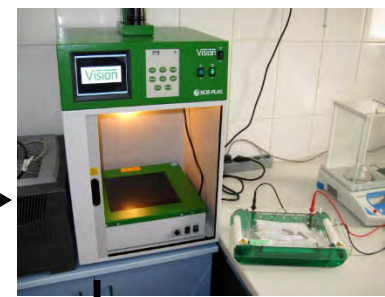
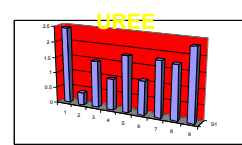
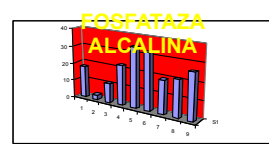
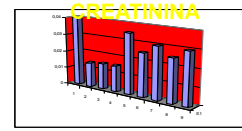
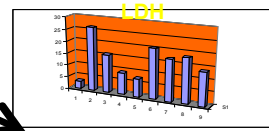
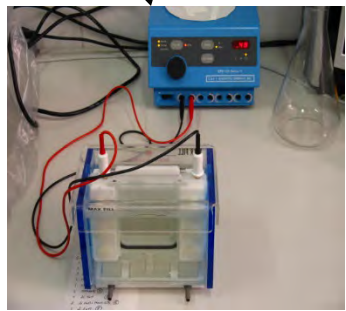
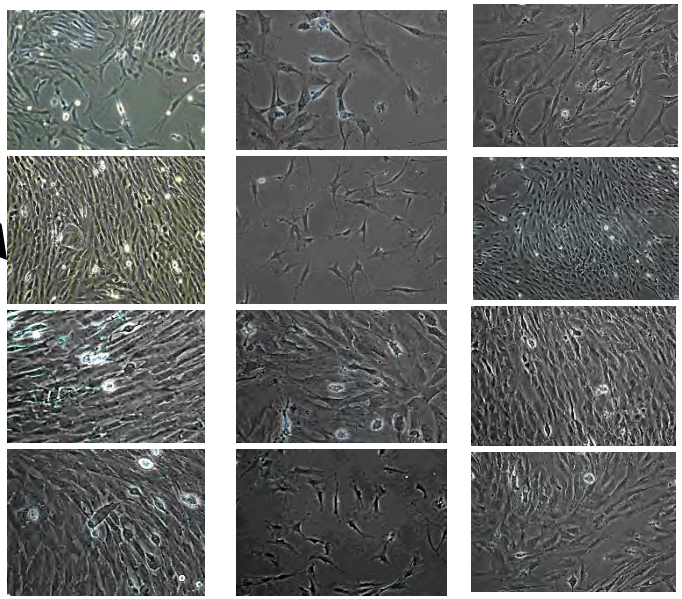
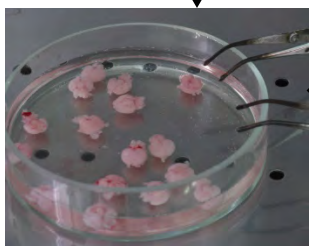


Analysis with GeneTools software v. 4 from SynGene of each track of the electrophoresis allowed us to compare the profiles of the total proteins expression.

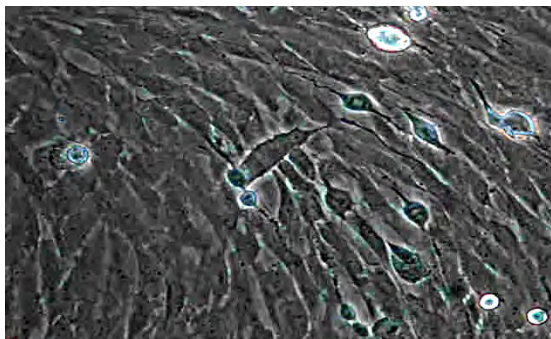
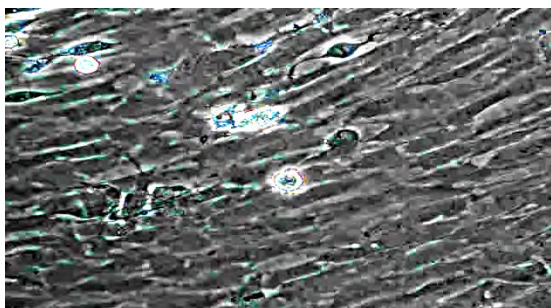
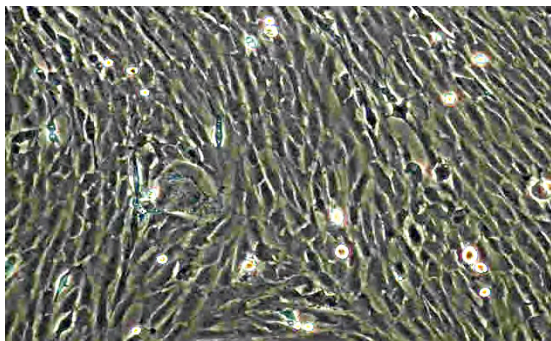
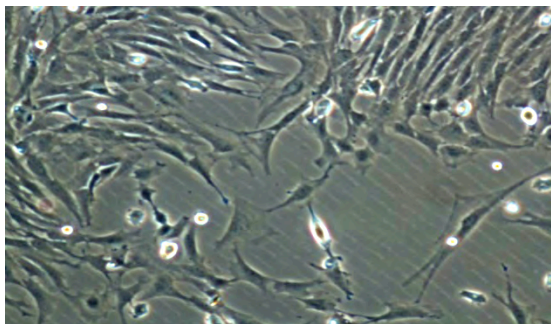




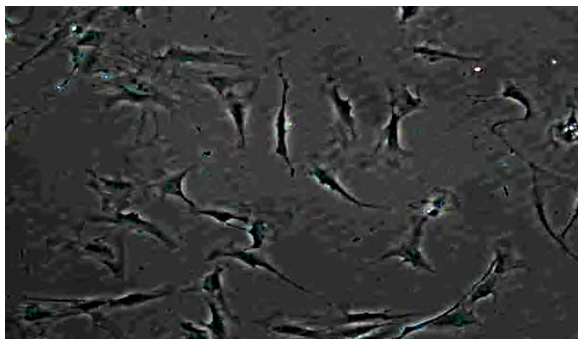
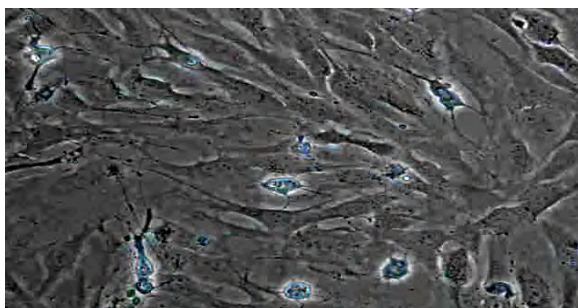
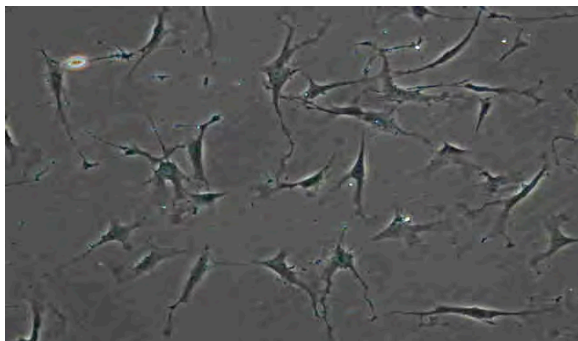
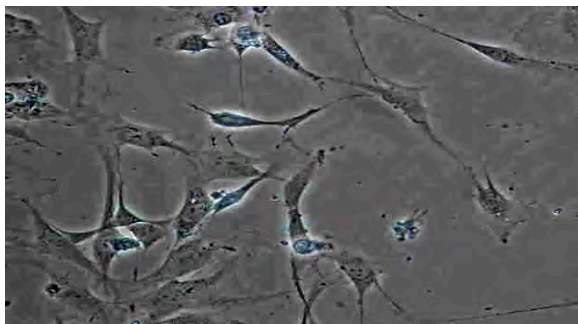




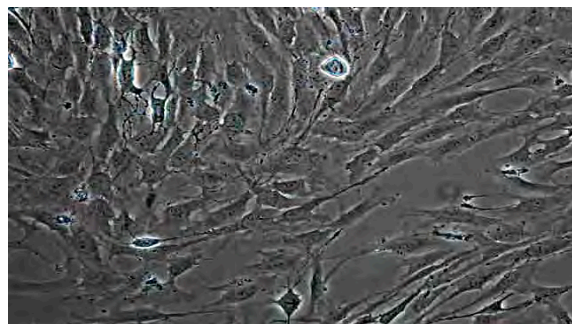
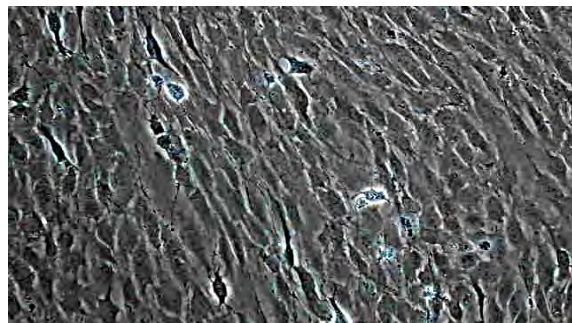
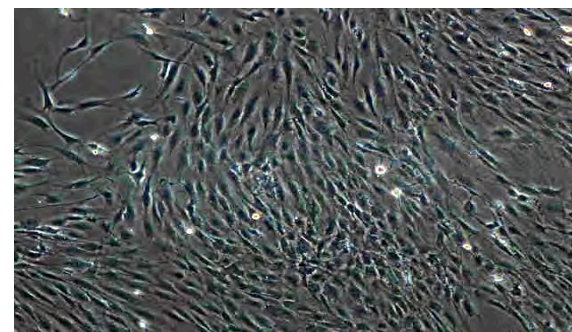
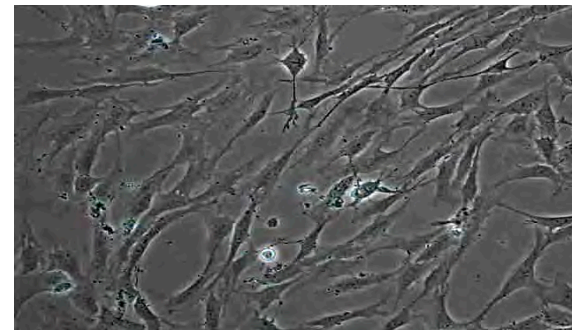
NORMALI (sanatosi clinic)



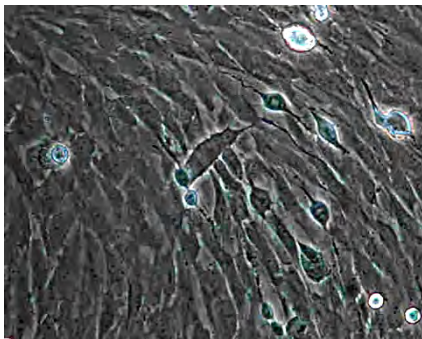
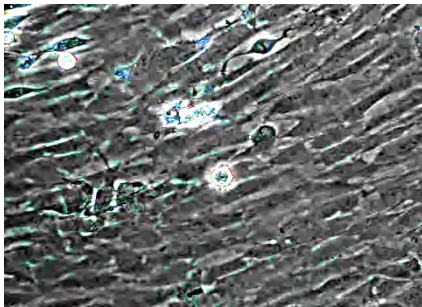
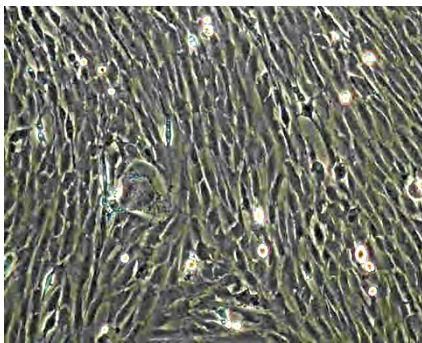
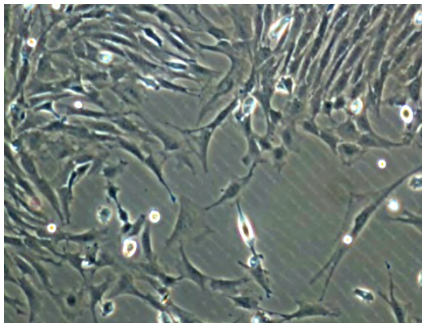
“ASMATICI (ovalbumina)”



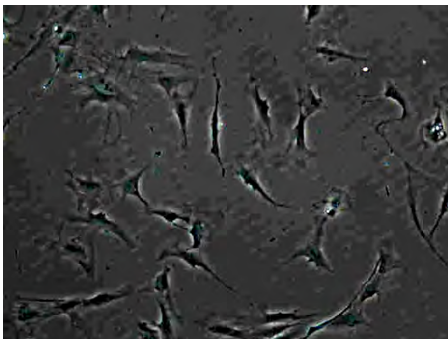
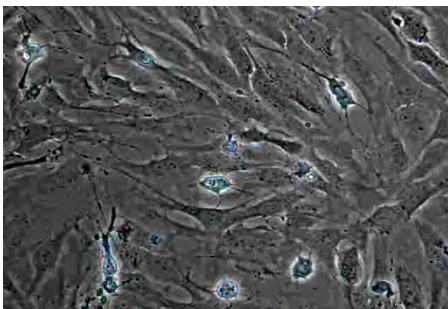
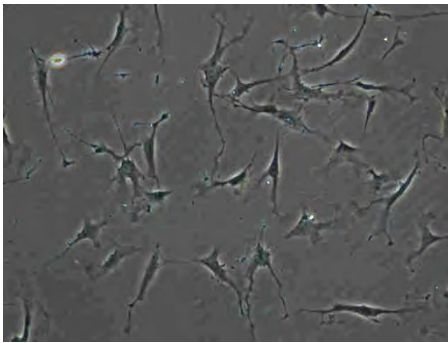
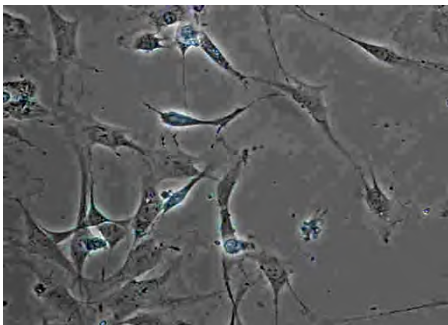
Asmatici trattati in Salina Turda



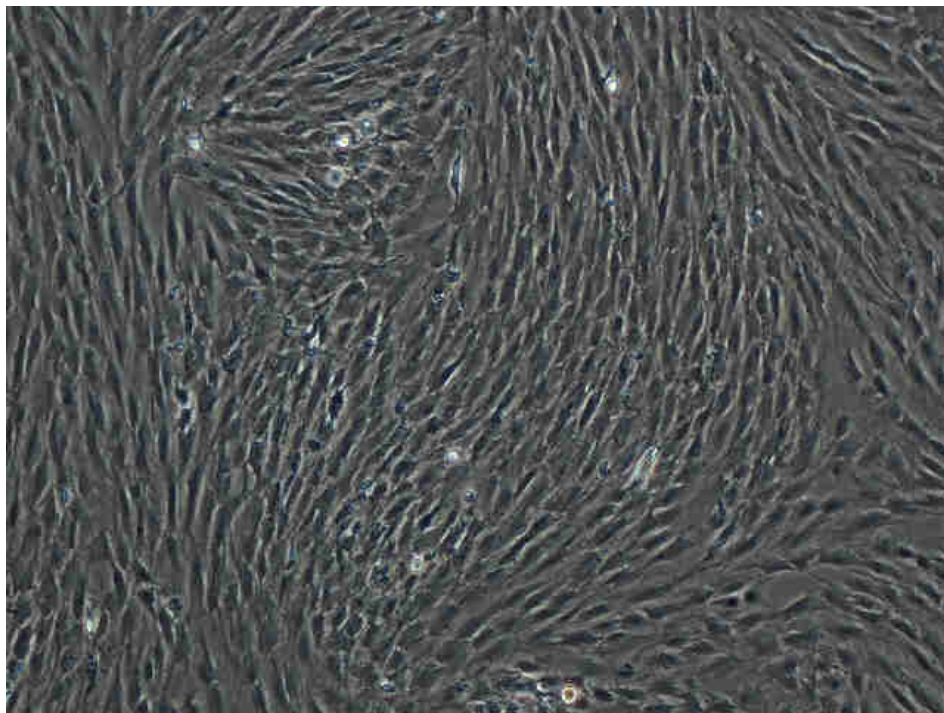
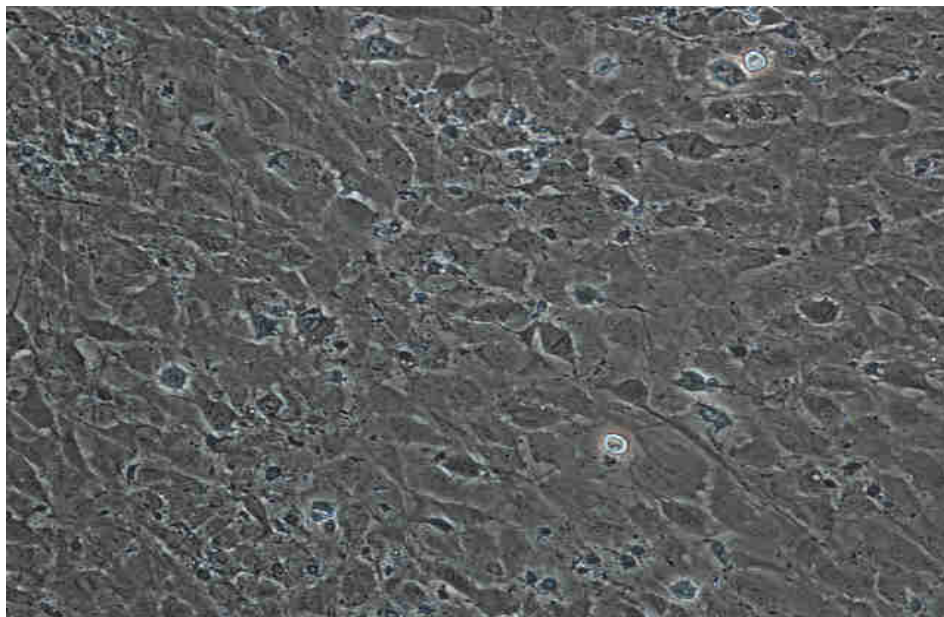
NORMALI (sanatosi clinic)



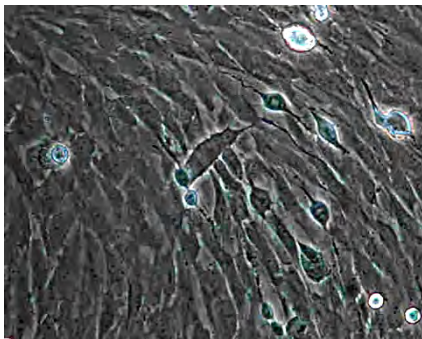
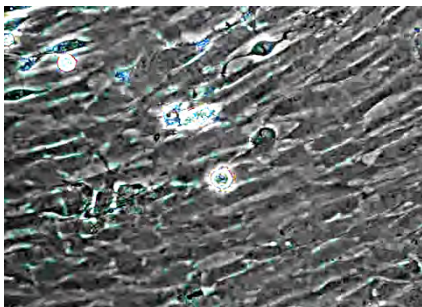
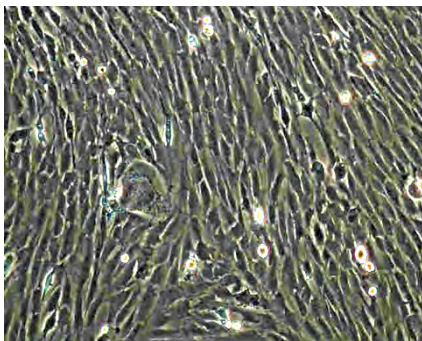
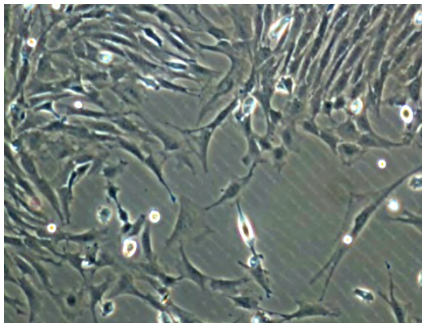
“ASMATICI (ovalbumina)”



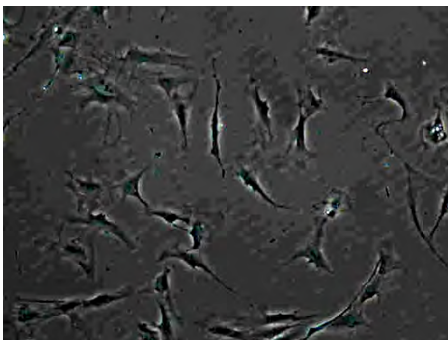
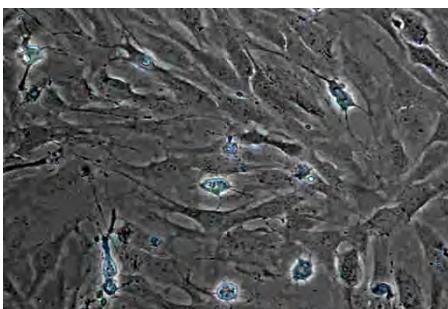
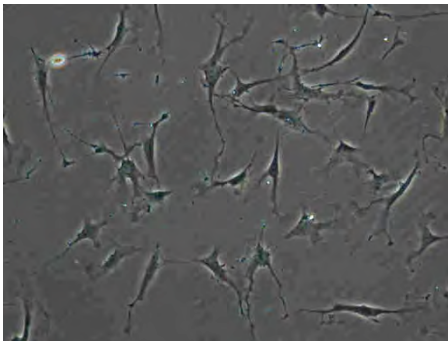
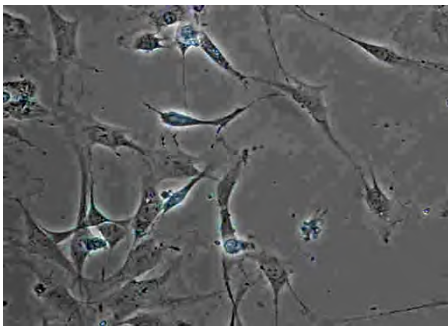
Asmatici trattati in Salina CACICA



NORMALI (sanatosi clinic)



“ASMATICI (ovalbumina)”



Asmatici trattati in Salina DEJ

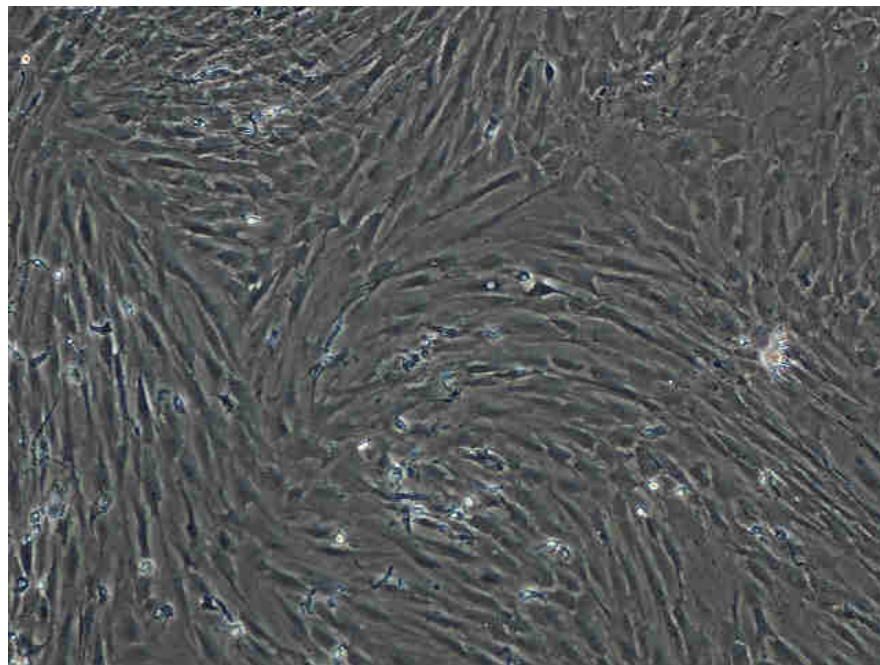
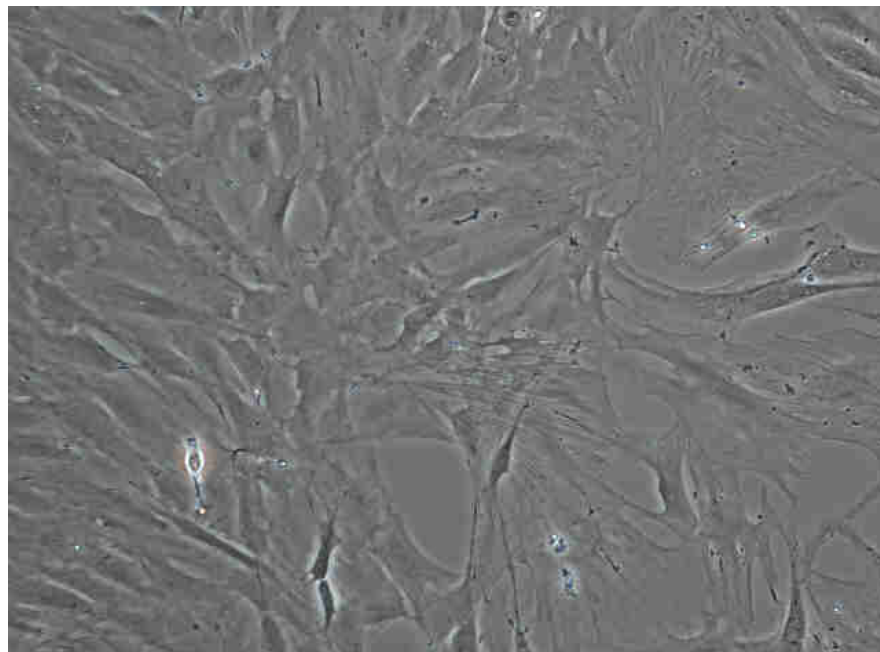


TABLE 1: SDS polyacrylamide gel electrophoresis of the pulmonary fibroblasts cultures

Samples	
5	9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats exposed to the saline medium of Dej Salt Mine
4	9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacic Salt Mine
3	9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats
2	9 days Control pulmonary fibroblasts culture
1	1- Sigma molecular markers

Fig. 1 – Electrophoretic profile of pulmonary fibroblasts cultures

PROBA	Cantitatea de proteine per placă
4- cultură de fibroblaste pulmonare expuse mediului din Salina Turda	155 µg
3- cultură de fibroblaste pulmonare obținută de la animale sensibilizate cu ovalbumină	130 µg
2- cultură de fibroblaste pulmonare maror	160 µg
1- markeri de greutate moleculară (SIGMA)	120 µg / 10 µl

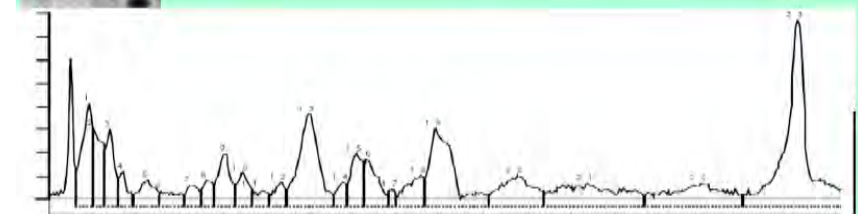


Fig.14 Densitograma omogenatului celular al culturii maror de fibroblaste pulmonare

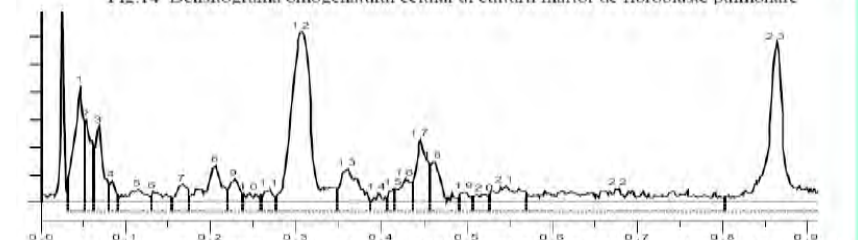


Fig. 15 Densitograma omogenatului celular al culturii de fibroblaste pulmonare obținută de la animale sensibilizate cu ovalbumina



Fig.16 Densitograma omogenatului celular al culturii de fibroblaste pulmonare obținute de la animale sensibilizate și expuse mediului din Salina Turda

Track 2 9 days Control pulmonary fibroblasts culture

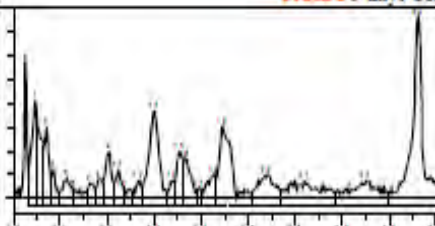


Fig. 2 Densitogram of 9 days Control pulmonary fibroblasts culture

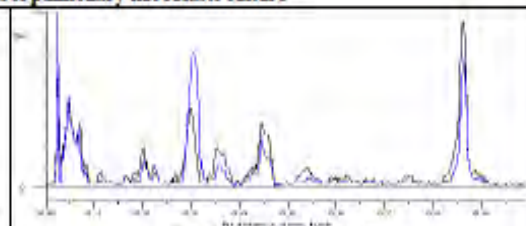


Fig. 6 Profile matching for CONTROL (—) - OVALBUMIN (—)

Track 3 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats

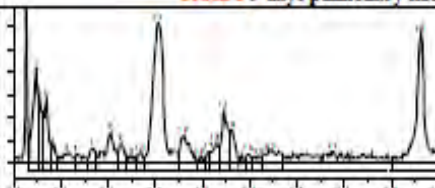


Fig. 3 Densitogram of 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats

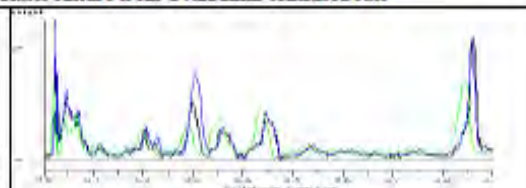


Fig. 7 Profile matching for CONTROL (—) - DEJ (—) - CACICA (—)

Track 4 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacic Salt Mine

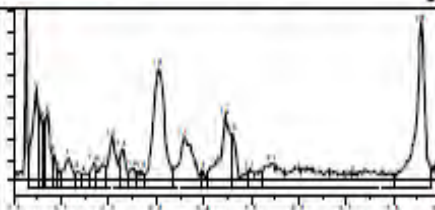


Fig. 4 Densitogram of 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacic Salt Mine

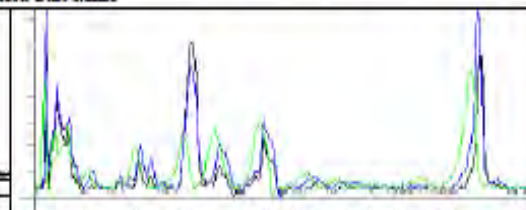


Fig. 8 Profile matching for OVALBUMIN (—) - DEJ (—) - CACICA (—)

Track 5 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats exposed to the saline medium of Dej Salt Mine

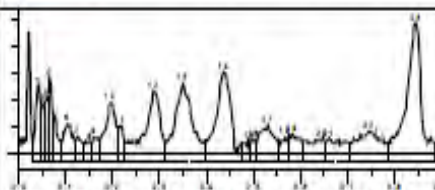


Fig. 5 Densitogram of 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats exposed to the saline medium of Dej Salt Mine

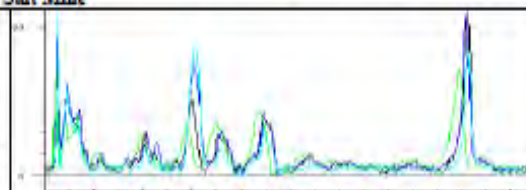


Fig. 9 Profile matching for CONTROL (—) OVALBUMIN (—) - DEJ (—) - CACICA (—)

TABLE 2 Protein expression analysis of the pulmonary fibroblasts cultures

Peak Nr.	Peak weights molecular limits (KDa)	CONTROL Quantity (µg/10µl)	OVALBUMIN Quantity (µg/10µl)	CACICA Quantity (µg/10µl)	DEJ Quantity (µg/10µl)
1	225 – 240	5,47	5,18	2,98	6,33
2	220 – 225	3,37	2,35	0,99	2,24
3	210 – 220	2,81	3,08	1,48	1,54
4	200 – 210	1,25	0,56	2,68	3,18
5	190 – 200	1,54	1,23	1,35	1,17
6	160 – 190	0,66	0,65	2,38	0,36
7	140 – 160	0,94	0,90	0,94	2,06
8	120 – 140	0,90	2,81	0,70	0,53
9	105 – 120	3,01	1,07	1,00	0,58
10	100 – 105	1,58	0,58	4,42	0,98
11	90 – 100	0,59	0,60	1,30	1,34
12	63 – 90	0,94	16,21	8,10	3,38
13	55 – 63	8,77	2,70	10,20	1,96
14	42 – 55	0,80	0,34	10,34	0,80
15	40 – 42	2,78	0,39	0,70	0,75
16	37 – 40	2,88	1,38	0,61	14,47
17	35 – 37	0,36	3,11	3,29	6,29
18	34 – 35	2,16	2,16	1,19	0,53
19	32 – 34	8,48	0,44	1,64	7,62
20	30 – 32	3,79	0,55	2,17	2,39
21	23 – 30	4,78	1,86	2,05	1,35
22	19 – 23	4,16	6,64	4,64	12,93
23	6 – 19	18,64	12,62	16,80	15,94
TOTAL amount of proteins in 10 µl of sample:		80,66	67,41	81,95	88,72

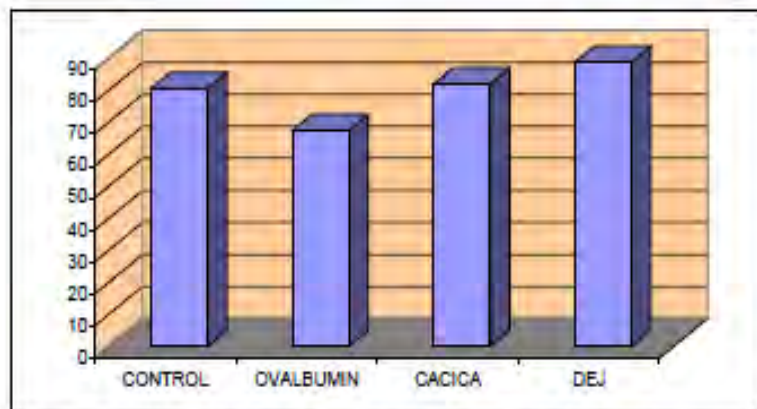
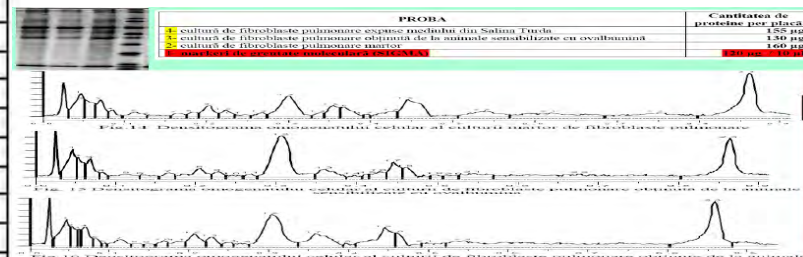
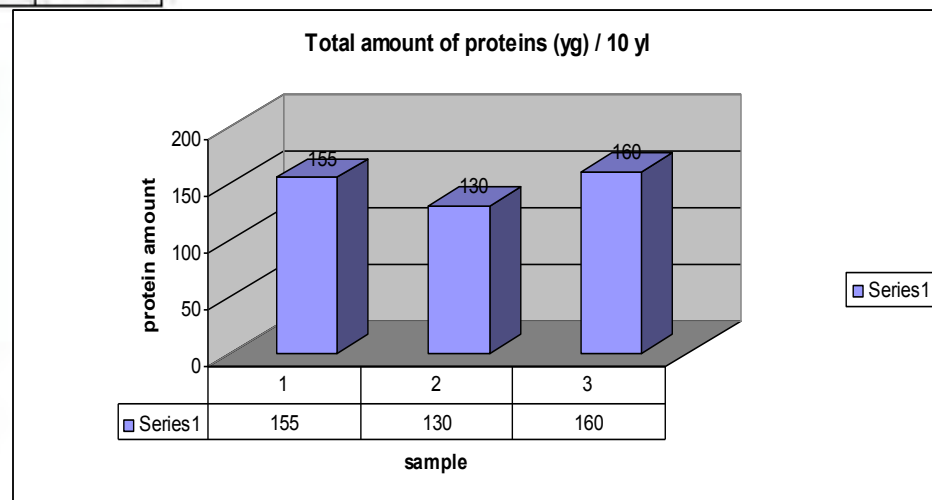


Fig.10 TOTAL amount of proteins in 10 µl of sample



Conclusions

Phase contrast microscopy analyses of primary fibroblasts cultures reveals an cellular regeneration after animal exposure to saline medium in Turda, Cacica and Dej Salt Mines, comparative with the cells morphology of cultures from Ovalbumin sensitised rats.

The morphological observations are confirmed by the electrophoretic analyses, which demonstrate through rising of the expression of many proteins and of total protein amount that the exposure of Ovalbumin-sensitised animals to the saline medium from Turda, Cacica and Dej Salt Mines is reversing the cells morphopathology of pulmonary fibroblasts in cultures;

Wistar rats sensitised with Ovalbumin have a low number pulmonary fibroblasts output cultures, with a more sensitive morphopatologic level.



Photo 1 Turda Salt Mine Entry

Munteanu C., Munteanu D., Simionca I., Cinteza D., Hoteteu M.;

Exploration of the speleotherapeutic potential through the cellular and molecular biology techniques**Abstract**

Objective: Exploring the speleotherapy effects on morphology and physiology of dermal and pulmonary fibroblast obtained from Wistar rats tissue in normal conditions and after induction of experimental "astma" awareness with ovalbumin.

**Medica - a Journal of Clinical Medicine**

ORIGINAL PAPERS: CLINICAL OR BASIC RESEARCH

Glial effects of the lithium mineral water Maria from Malnas-BaiConstantin MUNTEANU, MBIol^a, Gabriela ZAMFIRESCU, PhD^a, Diana MUNTEANU, MBIol^a, Delia CİNTEZA, MD PhD^b**ABSTRACT**

Objective: To investigate the influence of lithium mineral waters and lithium salts upon the differentiation of glial cells.

Material and methods: Mixed glial cultures were prepared from neonatal Wistar rat cortex. Cultures derived from neonatal rat forebrain develop with a monolayer or large flat astrocytes attached to the culture dish, with many smaller cells of the oligodendrocytes lineage on their surface.

Results: Treatment of these cultures with lithium mineral waters from Maria spring compared to treatment with lithium chloride 2mM showed significant differences in cell morphology. Immunohistochemical studies for glycogen synthase kinase (GSK-3 β) supported the protective effects of lithium mineral waters for glial cells, whereas lithium chloride 2mM determined cytotoxic effects and inhibited Wnt signalling pathway.

Conclusions: The results of this study indicate the fact that lithium chloride and lithium mineral waters induce changes in glial cells. The changes depend on the lithium level in the culture medium.

Key words: lithium, glial cells, GSK-3 β , GFAP, Laminin, Vimentin

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ANIONS	Content in 1 litre of mineral water				
	mg	mM	mlq	mg %	mlq %
Cl ⁻	1.009,1	28,460	28,460	10,922	24,866
Br ⁻	2,9	0,036	0,036	0,031	0,032
F ⁻	0,7	0,006	0,006	0,008	0,017
NO ₃ ⁻	9,9	0,160	0,160	0,107	0,139
SO ₄ ⁻	25,1	0,261	0,261	0,272	0,457
HCO ₃ ⁻	3.202,0	85,256	85,256	56,304	74,489
			114,455		100,000
CATIONS					
	mg	mM	mlq	mg %	mlq %
Na ⁺	2.263,8	98,441	98,441	24,503	66,009
K ⁺	70,5	1,803	1,803	0,763	1,575
Li ⁺	8,03	1,152	1,152	0,087	1,007
NH ₄ ⁺	0,70	0,039	0,039	0,008	0,034
Ca ²⁺	212,6	5,304	5,304	2,301	9,369
Mg ²⁺	38,3	1,164	1,164	0,306	2,031
Fe ²⁺	2,2	0,039	0,039	0,024	0,070
Mn ²⁺	0,1	0,002	0,002	0,001	0,001
			114,455		100,000
H ₂ SiO ₃	21,5	0,275		0,233	
HBO ₃	372,2	8,492		4,029	
NH ₃	7,0	0,437		0,076	
O ₂	2,4	0,130		0,026	
CO ₂	748,0	17,000			
Mineralization	9.239,0	231,477	228,909	100	

TABLE 1. Chemical content of Maria mineral water

Medica - a Journal of Clinical Medicine

ORIGINAL PAPERS

GSK-3 β expression after treatment of glial cells with lithium and Maria lithium mineral water from Malnas-BaiConstantin MUNTEANU, MBIol^a; Diana MUNTEANU, MBIol^a; Delia CİNTEZA, MD, PhD^b
^aNational Institute of Rehabilitation, Physical Medicine and Balneoclimatology, Bucharest, Romania
^b"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania**ABSTRACT**

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Conclusions: The results of this study indicate the fact that lithium chloride and lithium mineral waters induce changes on the expression of GSK-3 β .

Key words: glial cells, GSK-3 β , lithium, lithium mineral water, Malnas-Bai

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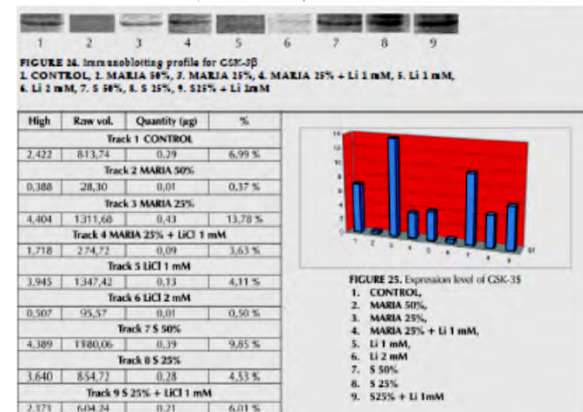


FIGURE 24. Immunoblotting profile for GSK-3 β
1. CONTROL, 2. MARIA 50%, 3. MARIA 50% + Li 1 mM, 4. MARIA 25% + Li 1 mM, 5. Li 1 mM, 6. Li 2 mM, 7. 5 50%, 8. 5 25%, 9. 5 125% + Li 1 mM

FIGURE 25. Expression level of GSK-3 β
1. CONTROL,
2. MARIA 50%,
3. MARIA 25%,
4. MARIA 25% + Li 1 mM,
5. Li 1 mM,
6. Li 2 mM,
7. 5 50%,
8. 5 25%,
9. 5 125% + Li 1 mM

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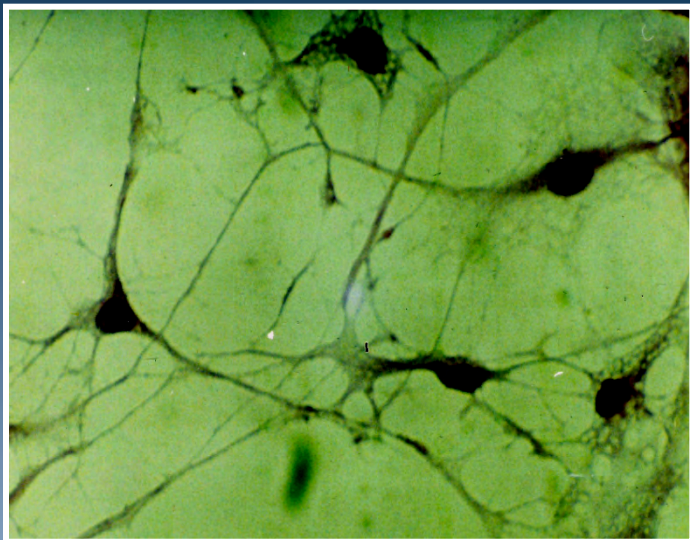
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Prof. Dr. Gelu Onose

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Dr. biol. Constantin Munteanu

E-mail: office@bioclima.ro
Website: bioclima.ro



THANK YOU !



www.bioclima.ro
Email: office@bioclima.ro



Constantin Munteanu