Morphological and electrophoretic data about heterogeneous primary skin cells cultures obtained from normal and Ovalbumin-Challenged Wistar rats after treatment by speleotherapy in the Cacica and Dej Romanian Salt Mines

Constantin Munteanu, PhD, [1a] Diana Munteanu, MBiol [a], Iuri Simionca, PhD [a], Mihai Hoteteu, [a]

[(a)] National Institute of Rehabilitation, Physical Medicine and Balneoclimatology, Bucharest, Romania

Abstract

Objective: To investigate the influence of salt mine medium from the Romanian Cacica and Dej Salt Mines upon the cell morphology and electrophoretic expression of heterogeneous skin cell cultures obtained from Wistar rats' abdominal skin, on normal and Ovalbumin-sensitized animals.

Materials and methods: Heterogeneous skin cell cultures were prepared from Wistar abdominal skin. Cultures derived from skin rat develop with a monolayer of fibroblasts and epithelial cells attached to the culture dish. Before cultures initiation, 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 Cacica and Dej Salt Mine for 14 days and maintained in the salt mine medium, as in speleotherapy treatment.

Results: Speleotherapy of Wistar rats had induced significant differences in cell morphology and electrophoretic expression of primary dermal cells cultures. The data obtained support the protective effects of speleotherapy by comparing with ovalbumin sensibilised animals.

Conclusions: The results of this study indicate the fact that speleotherapy induces changes on the morphology and protein expression of dermal cells in *vitro*, and these changes support the beneficial effects of speleotherapy.

Key words: speleotherapy, dermal cells culture, salt mine

[1] Corresponding author: Constantin Munteanu, tel./fax: +40213186458, e-mail: constantin2378@yahoo.com, mailing address: B-dul Ion Mihalache, nr.11A, District 1, cod 79173, Bucharest, Romania,
http://www.cell-culture.xhost.ro/

INTRODUCTON

Primary cell cultures can readily be obtained from human and animal skin using the explant method or trypsynisation.

Full thickness skin, also called the integument, is a composite of three tissues (epidermis, dermis and subcutaneous tissue), none of which constitutes a homogenous entity. Epidermis normally is composed of keratinocytes, which represent the largest population numerically, and lesser numbers of melanocytes, Langerhans'cells, and occasional cells of the lympho-reticular system, which are, however, transient members of the community.

Although the bulk of the dermis is noncellular (collagen and ground substance), within this compartment is also a variety of cell types, including fibroblasts, histicytes, mast cells, macrophages, lymphocytes and Schwann cells, endothelial cells of blood vessels and lymphatics, striated muscle cells of erector pili muscles, and smooth muscle of blood vessels.

The subcutaneous tissue includes most of the dermal cell types and fat cells as well (Flaxman, 1974).

The current study was designed to investigate the influence of salt mine medium from Cacica and Dej Salt Mines upon the cell morphology and electrophoretic expression of skin cells *in vitro* obtained from Wistar rats' skin, in normal and Ovalbumin challenged conditions.

Using skin cell cultures to verify the therapeutic properties of saline mines medium, described as speleotherapy, represents an innovative and scientific new way to establish the medical methodology of preventing, treating and recovery of patients with various dermatological problems.

MATERIALS AND METHODS

Materials

Phosphate Buffer Solution (PBS: NaCl 0,13M + KCl 2,6mM + Na_2HPO_4 x12 H_2O 8mM + KH_2PO_4 1,4mM); HAM-F12 culture medium (Sigma); penicillin 100 U/ml, streptomycin 100µg/ml; fetal bovine serum (Sigma).

Rat Wistar Model of Allergic Asthma
Wistar rats of 75-100g weights were sensitized to Ovalbumin by subcutaneous injections with 100 µg of Ovalbumin.

Primary fibroblasts culture

After anaesthesia with chloroform, rats were killed. After hair removing, a patch of 1 cm^2 of skin was detached en bloc in a laminar flow hood using sterile technique and put into ice-cold sterile Phosphate Buffer Solution (PBS: NaCl 0,13M + KCl 2,6mM + Na_2HPO_4 x12 H_2O 8mM + KH_2PO_4 1,4mM). 1mm tissue pieces were suspended in 0.125% trypsin and 0.001% DNase and repeatedly stirred for 6 minutes and centrifuged at 1000g. The pellet was resuspended in **HAM-F12** medium with 4500mg/l glucose, 25 mM HEPES, 100 U/ml penicillin, 100 µg/ml streptomycin and 50 µg/ml neomycin and 10% fetal bovine serum.

Phase Contrast Microscopy

Phase contrast microscopy, first described in 1934 by Dutch physicist Frits Zernike, is a contrast-enhancing optical technique that can be utilized to produce high-contrast images of transparent specimens, such as living cells (usually in culture), microorganisms, thin tissue slices, lithographic patterns, fibers, latex dispersions, glass fragments, and subcellular particles (including nuclei and other organelles).

SDS-PAGE Electrophoresis

The proteins electrophoresis from the total homogenate has as the purpose to establish the changes, which are revealed at the proteic level of skin cells cultures obtained from rats held on saline mine medium for the speleotherapy.

The proteins electrophoresis in gel of polyacrylamide was done in the denaturated conditions in the conformity with the techniques described by Laemmli (1979). The cultures have been washed with PBS, curetted from the culture plate and lyzed in buffer containing 0,5M Tris-HCl, pH 6,8 + 0,05% BPB + 10% glycerol + SDS 10%.

RESULTS

Control skin cells culture of 7 days has a heterogenic aspect with a high pre-confluence level. The cell division is to a high level and the cell morphology shows a typical microscopic view, described in the specific literature (fig.11). There are two types of cells: epithelial and fibroblastic.

Skin cells cultures of 7 days obtained from Ovalbumin sensitized rats presents many morphological changes from the control skin cell culture, being observed an sensible number reducing of dermal fibroblasts in culture, the diminished cellular dividing frequency and an

accentuated cellular morphopathology of the cells in culture. After 7 days of culturing, the pre-confluence level is much lower than in the control case (fig.12).

Skin cells cultures of 7 days obtained from Ovalbumin sensitized rats and treated by speleotherapy in Cacica Salt Mine shows an improvement of the morphological parameters of the cells comparative with the cultures obtained from Ovalbumin-challenged rats. By phase contrast microscopy, it is possible to observe a rising of the cells number (fig.13).

Skin cells cultures of 7 days obtained from Ovalbumin sensitized and treated by speleotherapy in Dej Salt Mine shows also an improvement of the morphological parameters of the cells comparative with the cultures obtained from Ovalbumin-challenged rats. It is observed the rising of the cell population density and that of cell viability (fig.14).

Skin cells cultures were homogenized with Laemmli buffer pH 6,8, and the proteins of the obtained homogenate were separated by 10 % SDS polyacrylamide gel electrophoresis that maintains polypeptides in a denatured state once they have been treated with strong reducing agents to remove secondary and tertiary structure.

Samples of 10µl were loaded into *wells* in the gel. One lane was reserved for Sigma molecular markers mixture of 205; 116; 97; 66; 55; 45; 36; 29; 24; 20,1; 14,2 and 6,5 KDa

Following electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250, that allowed visualization of the separated proteins. After staining, different proteins appeared as distinct bands within the gel (Towbin *et al.,* 1979).

Analysis with GeneTools version 4 software from SynGene of each track of the electrophoresis (fig.1), allowed us to compare the profiles of the total proteins expression (fig. 2- fig.10)

DISCUSSION

The present study evaluated morphological phenotypes related to repair and remodeling in fibroblasts and epithelial cells obtained from control Wistar rats and from Ovalbumin-sensitized and -challenged rats.

Compared with control culture, skin cells cultures from Ovalbumin-sensitized rats and Ovalbumin-sensitized treated in Cacica and Dej Salt Mines rats demonstrated the positive role of the saline medium for the sensitized rats.

The current study focused on skin cells, which are believed to play a major role in the organism – environment interaction. In this context, fibroblasts are believed to play a key role in maintaining and altering tissue structure. The ability of fibroblasts to migrate in response to chemotactic stimuli and to proliferate in response to specific growth factors is believed to control their accumulation at sites undergoing tissue repair. The ability of fibroblasts to produce and remodel extracellular matrix is thought to contribute to tissue structural changes. Remodeling of tissues likely involves fibroblast contractile activity.

In summary, the present study supports the concept that phenotypically altered fibroblasts can contribute to lesion repair in dermatological problems. Cells cultured from the skin of chronically OVA-sensitized and -challenged animals demonstrated consistently augmented repair responses for a number of functional assays (Sugiura *et al.*, 2007).

CONCLUSIONS

- Phase contrast microscopy analyses of primary skin cells cultures reveals an cellular regeneration after animal exposure to saline medium in 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 Cacica and Dej Salt Mines is reversing the cells morphopathology of skin cells in cultures;
- Wistar rats sensitised with Ovalbumin have a low number fibroblasts in skin cells cultures, with a more sensitive morphopatologic level.

Acknowledgments

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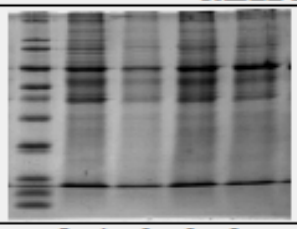
TABLE 1: SDS polyacrylamide gel electrophoresis of the dermal cells cultures



Samples

1- 7 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Dej Salt Mine

2- 7 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacica Salt Mine

3- 7 days skin cells culture from Ovalbumin-sensitised rats

4- 7 days Control skin cells culture

5- Sigma molecular markers

Fig. 1 – Electrophoretic profile of skin cells cultures

Track 4 7 days Control skin cells culture

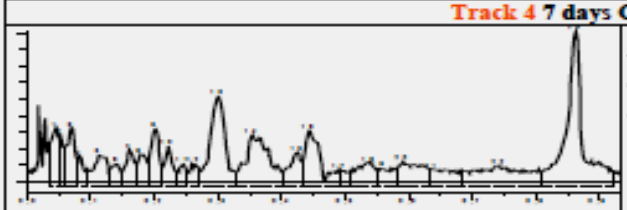


Fig. 2 Densitogram of 7 days Control skin cells culture

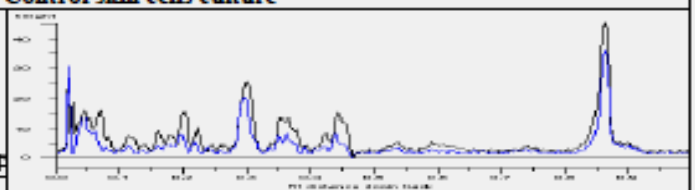


Fig. 6 Profile matching for CONTROL ( ) – OVALBIMN ( )

Track 3 9 days skin cells culture from Ovalbumin-sensitised rats

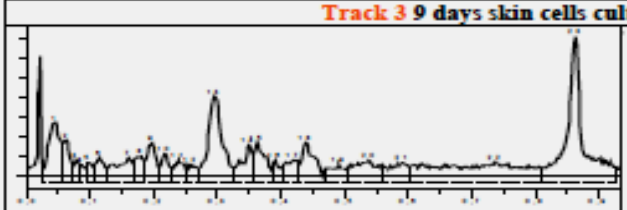


Fig. 3 Densitogram of 7 days skin cells culture from Ovalbumin-sensitised rats

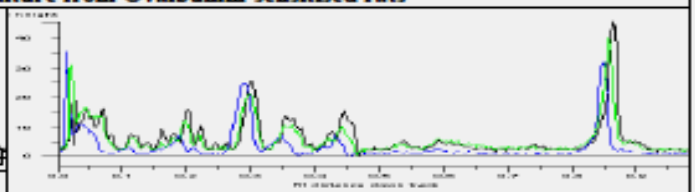


Fig. 7 Profile matching for CONTROL ( ) - DEJ ( ) - CACICA( )

Track 2 9 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacica Salt Mine

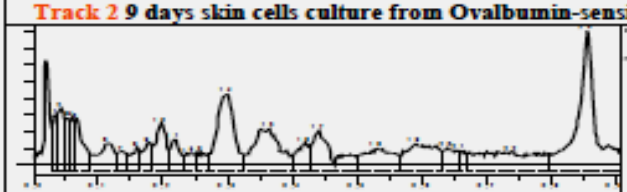


Fig. 4 Densitogram of 7 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacica Salt Mine

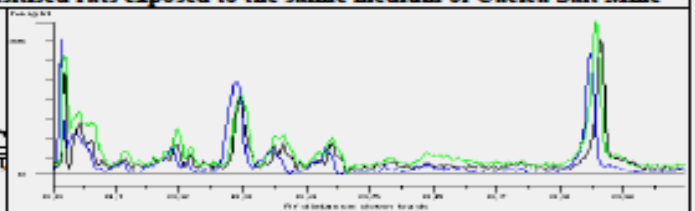


Fig. 8 Profile matching for OVALBUMIN ( ) - DEJ ( ) - CACICA( )

Track 1 9 days dermal cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Dej Salt Mine

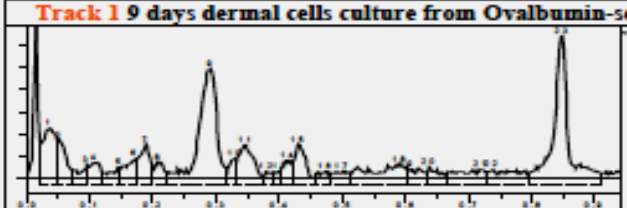


Fig. 5 Densitogram of 7 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Dej Salt Mine

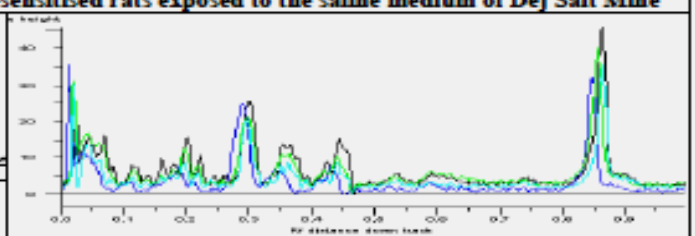


Fig. 9 Profile matching for CONTROL ( ) OVALBUMIN ( ) - DEJ ( ) - CACICA( )

TABLE 2 Protein expression analysis of the skin cells 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	3,80	5,61	2,60	5,56
2	220 – 225	1,81	2,19	3,58	2,19
3	210 – 220	4,78	0,92	2,07	0,92
4	200 – 210	1,66	0,37	2,04	1,25
5	190 – 200	3,55	0,80	3,86	0,80
6	160 – 190	1,61	1,35	3,39	1,86
7	140 – 160	2,81	2,73	1,12	2,50
8	120– 140	2,53	1,59	1,56	1,13
9	105 – 120	4,73	2,61	2,00	20,03
10	100 –105	2,75	1,45	4,28	1,02
11	90 – 100	1,32	1,36	2,45	3,77
12	63 – 90	1,61	0,90	1,21	0,29
13	55 – 63	13,14	9,93	1,36	0,34
14	42 –55	11,32	2,75	12,25	1,29
15	40 – 42	3,48	3,20	9,62	2,72
16	37 – 40	8,12	0,92	2,72	0,56
17	35 – 37	1,01	1,76	6,35	0,72
18	34 – 35	3,70	3,65	4,73	3,22
19	32 – 34	1,96	1,41	6,55	1,08
20	30 – 32	4,89	3,30	2,28	0,87
21	23 – 30	3,19	2,08	0,86	1,62
22	19 – 23	8,00	10,27	8,11	1,78
23	6 – 19	24,72	18,30	23,20	30,22
TOTAL amount of proteins in 10 µl of sample:		**116,5**	**79,45**	**108,19**	**85,74**

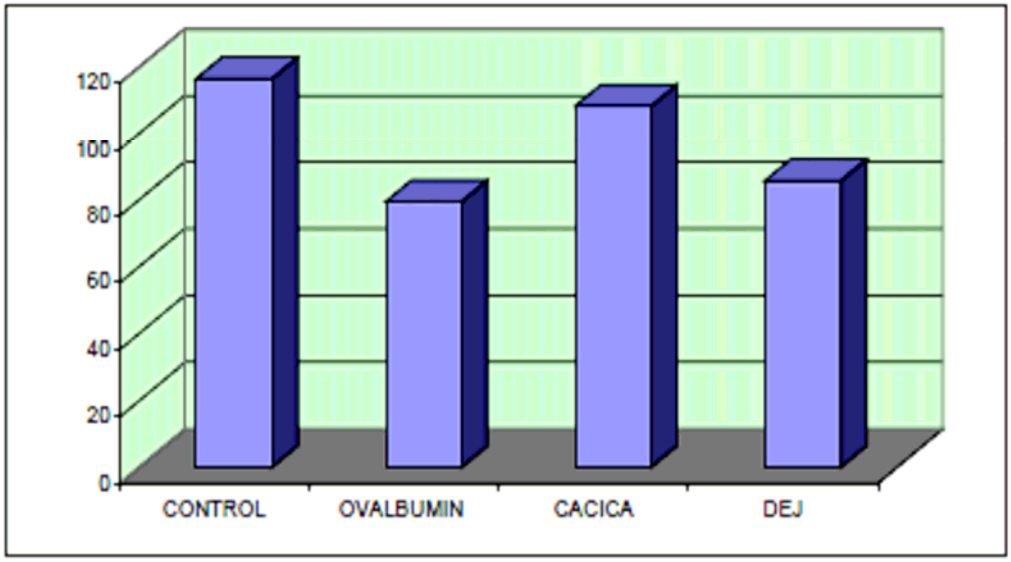


Fig.10 **TOTAL amount of proteins in 10 µl of sample**

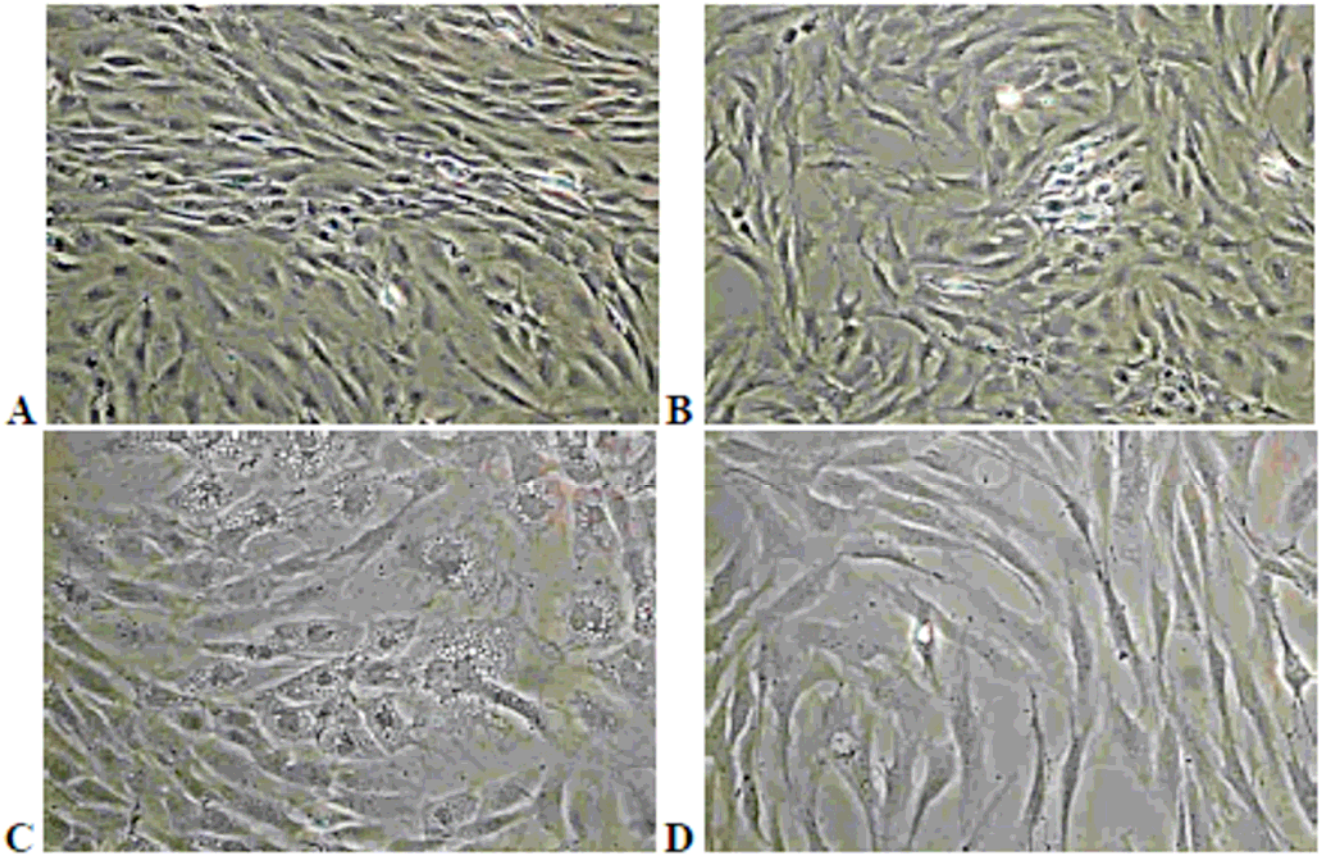


Fig. 11 Control skin cells culture of 7 days, A-B X 150, C-D X 300

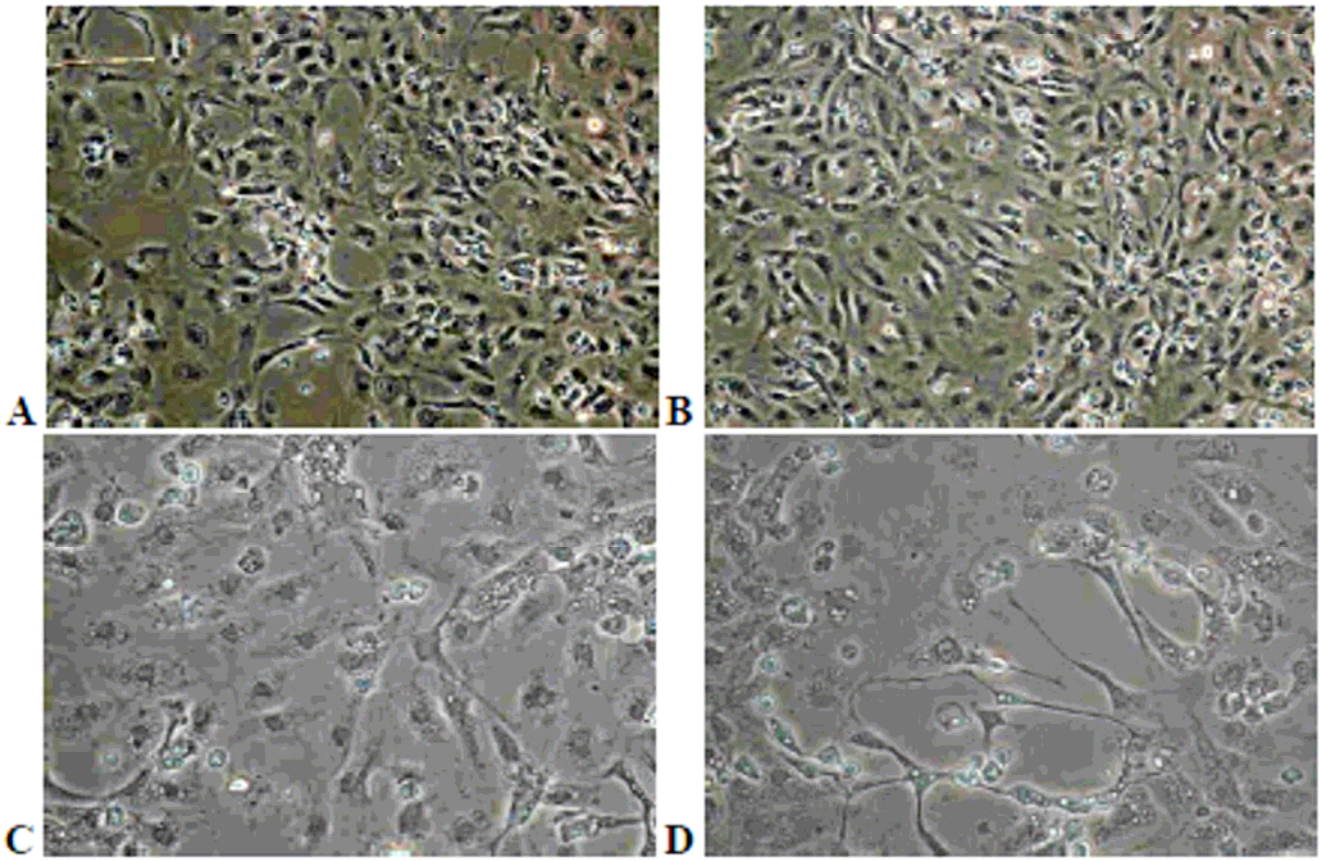


Fig. 12 7 days skin cells culture from Ovalbumin-sensitised rats, A-B X 150, C-D X 300

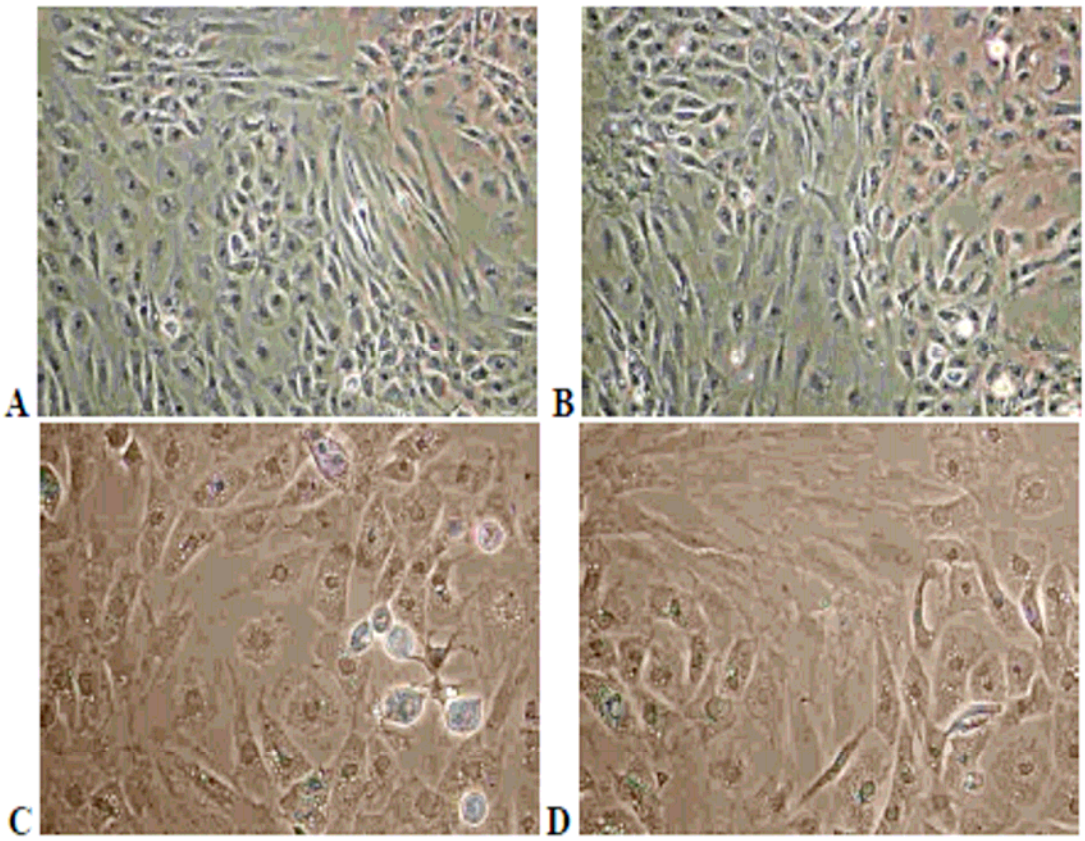


Fig. 13 7 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacica Salt Mine, A-B X 150, C-D X 300

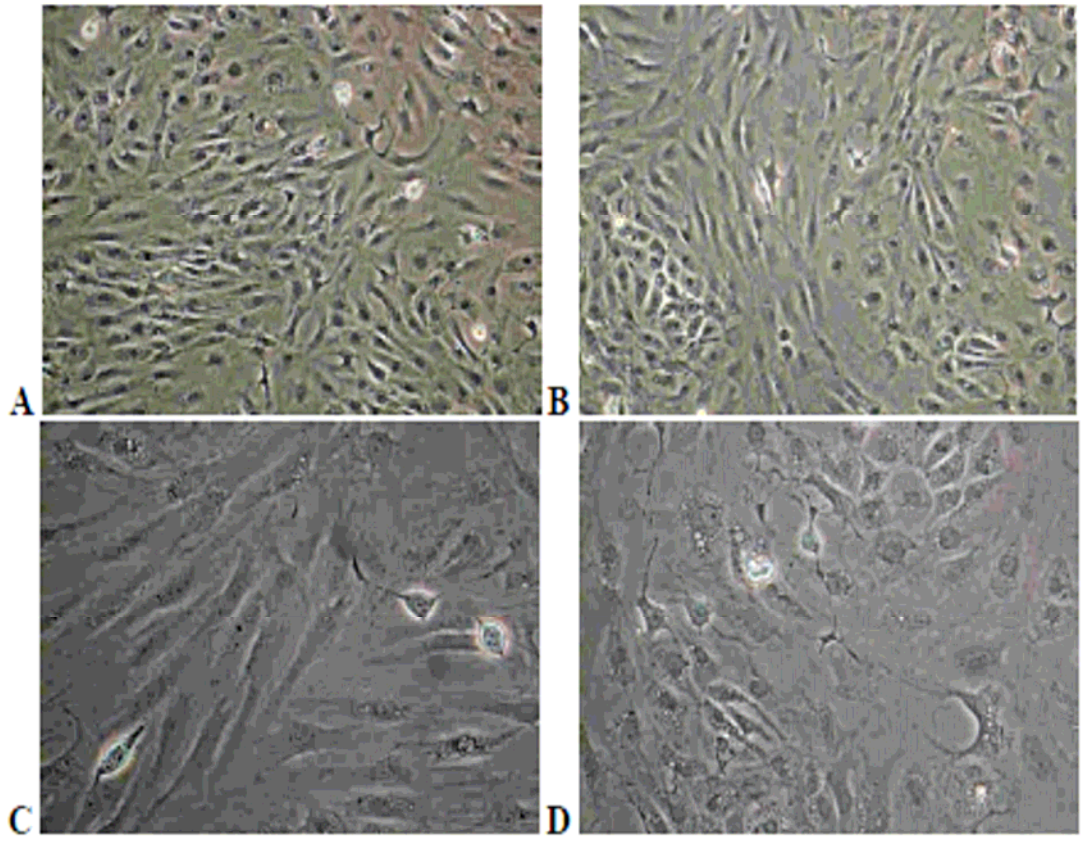


Fig. 14 7 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Dej Salt Mine, A-B X 150, C-D X 300