

THE RESTORATION OF SOME BICHEMICAL AND IMMUNOLOGICAL PARAMETERS AFTER THE ADMINISTRATION OF ECHINACEA EXTRACTS ON THE WISTAR RATS, CONSECUTIVE CYCLOPHOSPHAMIDE THERAPY

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ABSTRACT. Cytostatic action, although complex is directed upon divided and young cells, so that, they will especially influence organs and tissues where intense cellular proliferation processes occur. In this paper, we follow both some biochemical and immunological parameters changes after cyclophosphamide administration and rebuilding of these indices after 10 days of Echinacea extracts administration. The administration of the Echinacea extracts on rats already treated with cyclophosphamide has determined a partial restoration of the seric biochemical parameters. The restoration of the normal levels of the leucocytes treated with Echinacea extracts compared with the group treated only with cytostatics, shows the restart of the sanguine process of refilling with figurative elements, mostly as a result of the granulocytopenia, lymphocytopenia and monocytopenia stimulation at the level of hematogen marrow under the vegetal extract action.

Keywords: cyclophosphamide, *Echinacea* extracts, biochemical and immunological parameters

INTRODUCTION

In common life, in the extrauterine life, the bone marrow is the only stem cells supplier which can be distinguished from blood cells.

This differentiation with various blood types is finished for every erythrocytes, granulocytes, trombocytes within the bone marrow. Concerning lymphocytes, they are born within the marrow.

B lymphocytes, at mammals go on the processes of differentiation and maturation at the marrow level in comparison with birds where these processes are ceased in the Bursa of Fabricius, while T lymphocytes move to the thymus and they will get immune maturity. After this ceased processes T and B migrate to secondary lymphoid organs (lymph node, spleen, the Payer pates, etc.) which are organs representing lymphocytes site already distinguished matured immune competent were lymphocytes clonal spreading takes place under antigens influence. That is, the introduced organs assure occurring, differentiation, maturation and ceasing of those cells who participate at immune response, that is supply cells effectors of the immune system.

Assurance of a adequate number of such immune competent cells is crucial for a good occurring of defending processes.

Cytostatic action, although complex, is first directed to cells divided and to young cells, so that, they will especially act upon organs and tissues where intense cellular proliferation processes occur (Guest I et al, 2000; Hermenean A et al, 1995; Pasca C, 2000).

Echinacea plant extract has been used for immunostimulation for many years but the evidence supporting its therapeutic potential is still controversial. Many of its ingredients are powerful immune system stimulators.

Phytochemistry, pharmacology and clinical applications of *Echinacea* species, were the subject of more than 400 clinical studies, proving antiinflammatory properties (Tubaro et al, 1987), antibacterial (Steinmuller C et al, 1993; Bany J et al.,2003), antiviral (Egert D et al, 1992) and antitumoral (Miller S,2005) properties. The most valuable properties of extracts and isolated principles (especially polisaccharides) are bond to the non-specific stimulation of the immune system (Morazzoni P, 2005) through the stimulation of PMN and macrophage phagocytosis (Gaisbauer N et al, 1990), stimulation of TNF- α (Gertsch et al,2004), interleukine 1 (IL-1), interleukine 6 (IL-6) production by macrophages (Burger R et al, 1997), increased proliferation of T lymphocytes populations (Wildfeuer A et al,1994), and stimulation of NK cell activity (Brousseau M et al, 2005).

MATERIAL AND METHODS

Animals

The experiments were made on white male Wistar rats, weighting 170-180 g, kept under standard diet and conditions.

The following animal groups were used: control group (M); group treated with Cyclophosphamide (40 mg/kg body/day) administrated by intraperitoneal injections for 3 days; group treated with Cyclophosphamide for 3 days and with *E. pallida* extracts (5g/kg body/day) for 10 days, administered by intragastric gavage; group treated with Cyclophosphamide for 3 days and with *E. purpurea* extracts (5g/kg body/day) for 10 days.

The animals were sacrificed after finishing the treatment (14 days Cyclophosphamide first dose given).

Antitumoral drug

Jenapharm, Ankerwerk-Germany
Ciclofophosphamide (CFA), was before deluted in a 0,9% sterile physiologic Na Cl solution and intraperitoneal animals injected for 3 days.

Echinacea extracts

The *Echinacea* fluid extracts were got through 60° alcohol Squibb technique (Ionescu, 1977). There were determined polysaccharide in the *Echinacea* extracts after a spectrophotometry technique using anthrona – sulphuric acid coloured reactive (Tamas M, 1991) and phenyl- propane compounds were noted in caffeic acid. *Echinacea* extracts were 1% immunostimulatory polysaccharide standardized and 0,5% minimum propanic phenyl compound noted in caffeic acid.

Biochemical parameters

Seric cholesterol. It is about ser taken from Zlatkis-Zak method. The method principle is the following: cholesterol in concentrated icy acetic acid with the addition of ferric clorure calculation (FeCl₃) gives an intense pink-violet reaction which is read at the $\lambda=560$ nm Spekol.

Alcaline phosphatase is determinined from ser or tissular homogenate after Bergmayer' methods (1962) using pH=9 p-nitrophenil-phosphate and 37°C incubation.

Acid phosphatase. The method described by Bergmayer, keeps the same principle like alkaline

phosphatase, but the enzyme activity is got in an acid medium also using as substrate p-nitrophenil-phosphate. Results are also read at $\lambda=405$ nm Spekol and are expressed in mU/g tissue or /mg protein or U.E./l ser.

Butyl cholinesterases form ser or tissues are got after the Gorun method using butirilcoline iodine as substrat. Cholinesterase makes hydrolysis butyl choline making up thiocoline and butyric acic. The resulted thiocoline reacts together with DTNB making up a yellow compound and the extinction is red at $\lambda=405$ nm. The results are expressed in mU/g tissue or /mg protein or U.E./l ser.

Statistic processing of biochemical parameters

Statistic processing of biochemical parameters and enzymes watched in ser, blood and tissue homogenates, carried out cf. the „t” test .

RESULTS AND DISCUSSION

Regarding biochemical parameters at CFA group, these have lower values than the control (table 1). The seric cholesterol at animals from CFA treated group is 12,7% decreased in comparison with the control.

No significant modification has been recorded regarding this parameter after the administration of the *Echinacea* extracts in neither of the groups, the level of CFA remains lowered at both groups (14,76% and 13,65%). The alkaline phosphatase activity is 20,81% decreased at CFA treated rats. The butyl cholinesterases activity is 33,05% lowered after CFA administration. For the group treated with *Echinacea purpurea* we have obtained values close to the control group and regarding to the group treated with *E. pallida*, significant increasing has been recorded. The butyl cholinesterases activity has been decreased with 33,05% after the CFA administration, but, on the other hand, the *Echinacea* extracts administration has determined a restoration of this enzyme activity on both groups, the low level variations being statistically not significant.

Table 1

EVOLUTION OF SOME BIOCHEMICAL SERIC PARAMETERS AT WISTAR RATS TREATED WITH:
CYCLOPHOSPHAMIDE (CFA), CFA+E. PALLIDA, CFA+E. PURPUREA

Parameters	Control	CFA	CFA+Epu	CFA+Epa
Cholesterol	271±6,18	238±6,24	234±4,61	231±±6,41
mg%	(7)	(7)	(7)	(7)
	-	-12,17	-13,65	-14,76
	-	P<0,01	p<0,01	p<0,001
Alcaline phosph.	58,6±21,81	46,37±2,13	35,84±0,95	41,24±1,24
mU/ml	(7)	(7)	(7)	(7)
	-	-20,89	-38,86	-29,64
	-	p<0,001	p<0,001	p<0,001
Acid phosph.	16,56±1,29	13,34±0,93	16,15±0,69	20,29±0,40

mU/ml	(7)	(7)	(7)	(6)
	-	-19,44	-2,47	+22,52
	-	p<0,05	p>0,5	p<0,02
BCE	4616±287	3090±205	4301±142	5155±390
mU/ml	(7)	(7)	(7)	(7)
	-	-33,05	-6,82	+11,66
	-	p<0,001	p>0,5	p>0,25

Having a close view under the leucocytes content at the groups treated with CFA, respective CFA and *Echinacea pallida*, we can notice that the CFA administration has determined a lowering of the neutrophils number level (fig.1) from the periferic blood beginning after 24 hours from the CFA administration. We can notice also a lowering of the lymphocytes number (fig. 2) and also of the monocytes (fig.3). After a 5-7 days period from the CFA

administration, the process of remaking and respreading of the sanguine elements has begun, and at the animal groups treated with *Echinacea* as a plus, these processes are restored at a higher level. These results lead us to the suggestion that the extract administration makes a stimulation effect at the hematogen marrow level, and the rebuilding processes are more accelerated then the natural ones.

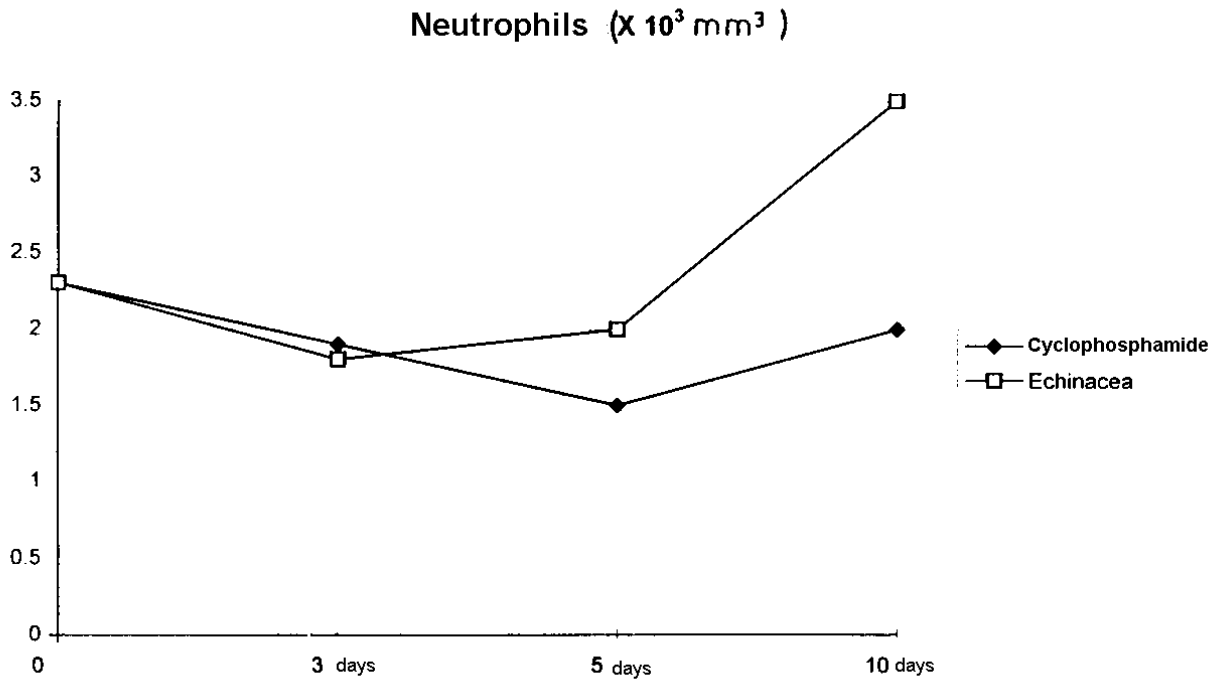


Fig.1 The variation of neutrophils at CFA and CFA + *E. pallida* groups

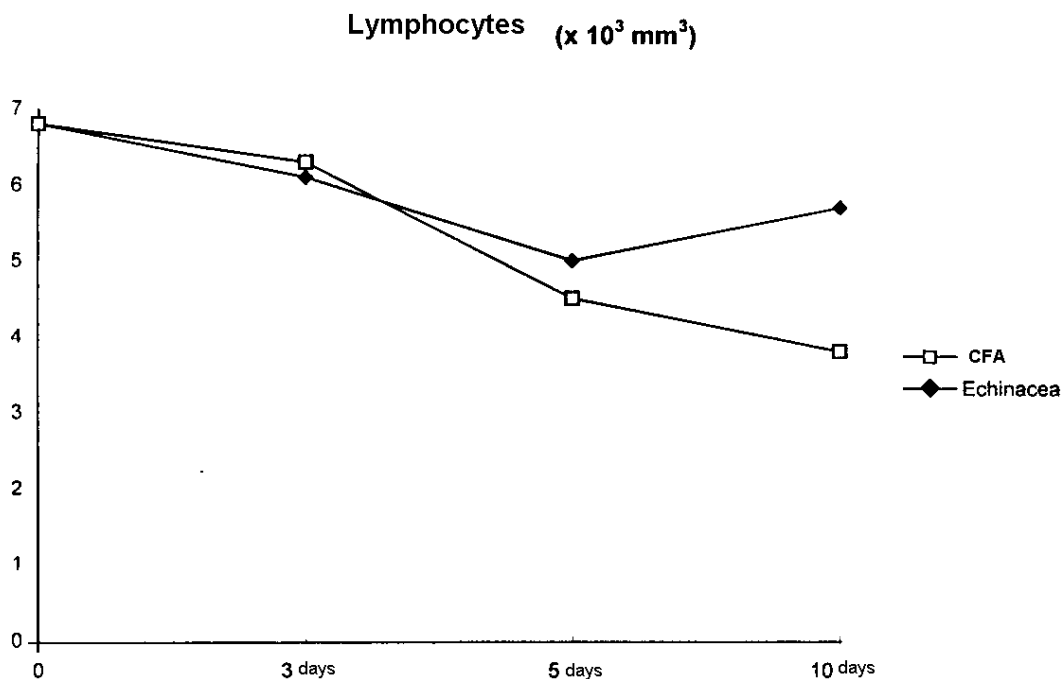


Fig. 2 The variation of neutrophils at CFA and CFA +*E. pallida* groups

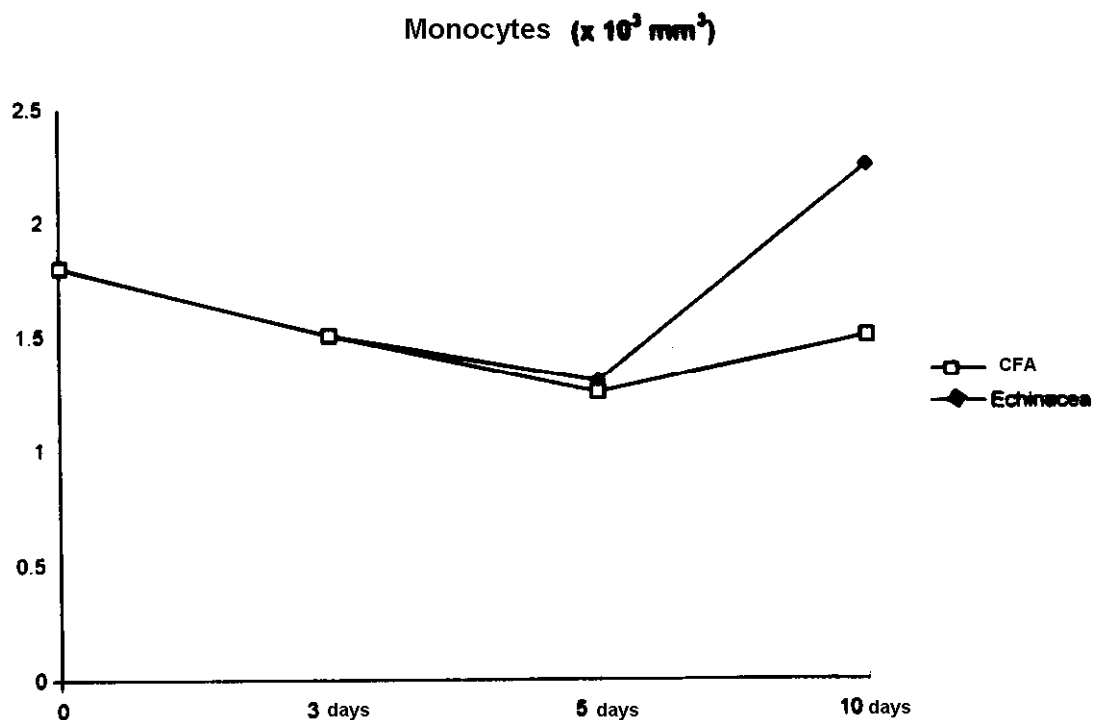


Fig. 3 The variation of monocytes at CFA and CFA +*E. pallida* group

CONCLUSIONS

The *Echinacea* extracts administration on the rats treated with cyclophosphamide has determined a partial restoration of the biochemical and immunological parameters. The restoration of the normal values of the leucocytes of the Wistar rats treated with *Echinacea* on behalf of the group treated with cytostatics only, shows us the restoring of the respreading process of the blood cells, probably due to the granulocytopenia, lymphocytopenia and monocytopenia stimulation at the level of hematogenous marrow under the vegetal extract action.

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