THE HAEMATOLOGICAL AND BIOCHEMICAL PROFILE OF THE TUMOR-BEARING RATS SUBMITED TO THE CYTOSTATIC TREATMENT WITH SOME POLYPHENOLIC OR POLYSACCHARIDIC BIOPREPARATIONS

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Abstract: The assessment of the biocompatibility between the development of the tumor-bearing animal’s physiological processes and the polysaccharidic or polyphenolic antitumoral treatment was performed *in vivo*, on experimental models adequate to the preclinical pharmacotoxicological research. The slight decrease of the total number of circulatory erythrocytes, – correlated, by sense and amplitude, to the levels of the hemoglobin and hematocrit – the low level of leukocytosis and trombocytosis or thrombocytopenia, have reflected a moderate impact of the polysaccharidic or polyphenolic extracts upon the hemo- , leuko-, and thrombopoetic organs. The plasmatic hypoproteinemia, hypoalbuminemia, hyperglobulinemia, moderate hypoglycemia, hypo- or hyperlactacidemia, minor hyperlipemia, hypocholesterolemia and the slight increases or decreases of the plasmatic free fatty acids levels - biochemical variations registered in our experimental conditions - suggest the interference of the bioactive agents with the tissular metabolic processes, which is materialized through modifications of some plasmatic biochemical parameters, which are circumscribable in the normal limits and not in the pathological ones. The bulk of results proves the moderate toxicity of the polysaccharidic and polyphenolic biopreparations upon the normal cells of the animal organism and, especially, upon the organs directly involved in maintaining the internal medium homeostasis.

INTRODUCTION

A major desideratum of nowadays oncochemotherapy is, on one hand, to perturb and/or to distroy the malignant cells – from the structure of the neoplasm detected at an early stage, or those remained postsurgically after surgical procedure– without affecting normal cellular processes, and on the other hand, to allow the host’s normal immunitary defense against the residual tumor cells. However, most chemotherapeutic agents used in the antineoplastic treatment are toxic at the efficient doses, immunosuppressive and cannot be used at high doses without affecting the host, altering the vital functions not only of the aggressive malignant cells, but also those of the normal cells from the invaded body.

Generally speaking, the administration of various anticancer drugs leads to the emergence of some side effects, the toxic reactions appearing at the level of different tissues and organs and taking the most various forms and intensities– from clinically unnoticeable to deadly. Thus, the necessity to achieve preclinical pharmacotoxicologic studies upon some new agents, proven to be cytostaticaly active, for the evaluation of their potential side effects. (Mellet, 1969; Medoff et al., 1974; Borsos et al., 1976; Bedeleanu and Kory, 1976; Cotrău, 1978; Balls and Bridges, 1984; Bonta et al., 1985; Borenfreund and Puerner, 1985; Calabresi and Parks, 1985; Goodman and Gilman, 1985; Ludlum, 1985; Chiricuţă, 1988; Ciudin and Marinescu, 1996; Salmon and Sartorelli, 1995; Stroescu, 1998; Miron, 2000; Devita, 2001;).

In this informational context, we remind that our group’s *in vitro* and *in vivo* previous investigations – on experimental models appropriate to the qualitative and quantitative pharmacodynamic evaluation – have highlighted, confirmed and quantified he antineoplastic effect of some polysaccharidic and polyphenolic autochthonous biopreparations, these bioactive compounds being preclinically characterized as new potential cytostatic agents (Rotinberg et al, 2008; Mihai et al, 2008). Thus were set the grounds for preliminary pharmacotoxicological studies, in order to establish the compatibility, or lack of it, between the polysaccharidic or polyphenolic antitumoral treatment and the development, within normal limits, of the physiological processes of the animal organism aggressed by neoplasm.

Thus, the present paper includes some of the results obtained during a partial and preliminary investigation of the cytotoxicity of the treatment with the polysaccharidic and polyphenolic extracts –administered in the effective cytostatic dose – upon the normal cells of the animal bearing Walker 256 carcinosarcoma.

MATERIAL AND METHODS

In the framework of *in vivo* tests there have been included the polysaccharidic fungal biopreparation EPzAgsp, obtained from *Agaricus* sp. through hydrous extraction, and the polyphenolic bioproducts coded EPfRc and EPfHr, which were obtained by alcoholic extraction from bark of *Rosa canina* and *Hippophae rhamnoides* shrubs.

In vivo testing of the polysaccaridic or polyphenolic chemotherapy cytotoxicity on the tumor bearing animals’

physiological processes has been performed on Wistar, white male rats of 165-180 g, with Walker 256 carcinosarcoma, of solid type. The trial animals were held in individual cages, in a normal light/darkness cycle, and a 22-23°C ambient temperature, having free access to water and standard food.

Twenty-four hours after the subcutaneous tumoral transplant, with 0.2 ml suspension of cancerous cells (Pollak&Fidler, 1982), in the case of Walker 256 tumor, the antimalig treatment was initiated and continued for 19 days. It was given through intraperitoneal (i.p.) daily injections of the polysaccahridic or polyphenolic biopreparations in dose of 30 or 20 mg/kg body weight, the doses used being antitumoral efficient. An equivalent volume of saline solution was administered to the control animals.

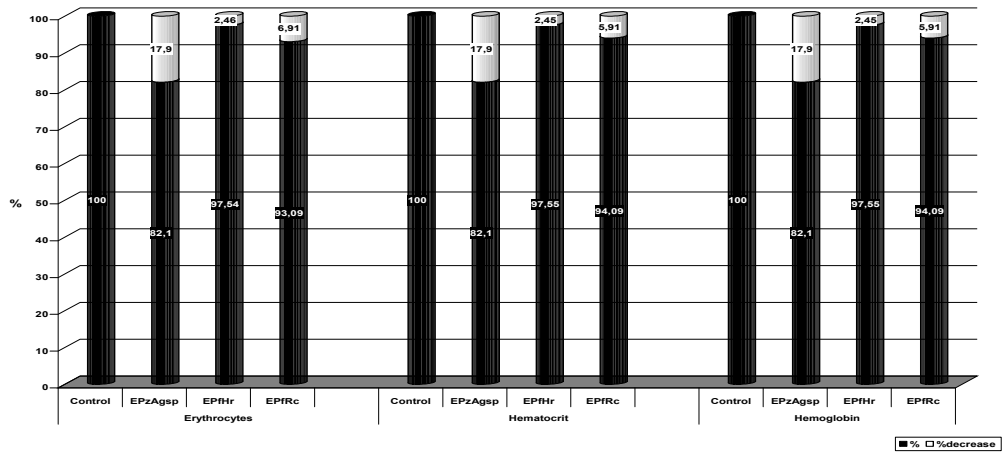
At the end of the treatment period, the experimental animals were killed by decapitation and the blood was prelevated in the presence of EDTA anticoagulant, in order to obtain the entire blood and plasma necessary to establish, on the one hand, the hematological picture - the total number of red blood cells, the average red cell volume, hemoglobinemia, hematocrit, the total number of white blood cells, leukocyte formula, total agranulocytes number (lymphocytes, monocytes) and granulocytes (neutrophils, basophils, eosinophils), total platelet count and, on the other hand, the plasmatic biochemical profile, built by the glycemy, lactacidemy, proteinemy, albuminemy, globulinemy, lipemy, cholesterolemia and free fatty acids values

The assessment of the haematological picture was made with the veterinary hematologic analyzer Melet Schloesing MS9, while the biochemical determinations of the plasma glucose, lacic acid, total proteins, albumin and globulins, total lipids, cholesterol and free fatty acids were made by spectrophotometrical specific methods, sometimes adapted. (Artenie and Tanase, 1981; Ciudin and Marinescu, 1996; Kondi, 1981; Nuta and Busneag, 1977)

The appreciation of the toxicity of the polysaccharidic or polyphenolic bioproduct was based on the statistical analysis of the values of the pharmacotoxicological effect evaluating indices, in the light of Student's "t" test (Snedecor, 1968) and on the comparative analysis of our results with the standard values set by the World Health Organization, Voicu (Voicu, 1997) and Gossel (Gossel and Douglas, 1994), for the classification of acute and subacute toxic effects of a drug treatment, in toxicity degrees (Paunescu-Podeanu, 1962; Traian et al, 1991).

RESULTS AND DISCUSSIONS

In vivo effect of EPzAgsp polysaccharidic extract and of the EPfRc and EPfHr polyphenolic biopreparations, administered in different doses to animals aggressed by Walker 256 carcinoma, upon some haematological parameters – graphically illustrated in Figure 1 – as compared



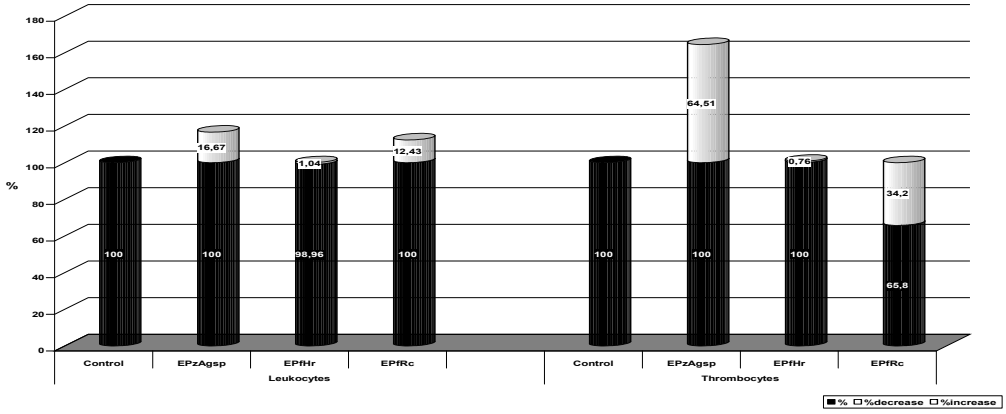


Fig. 1 The percentage variations of the average number of red blood cells, hemoglobin and hematocrit and of the mean number of circulating white blood cells and platelets, registered consequently to the daily intraperitoneal treatment with the fungal or vegetable biopreparations.

to the control groups, has conditioned:

- a moderate reduction in the number of circulating erythrocytes (17.9%), of the hematocrit (17.9%) and haemoglobin (22.8%) - indices that are correlated, in sense and amplitude, with the erythrocytes numerical variations - in the case of the polysaccharidic treatment. As regard to the polyphenolic extracts, the percentage variations of the three indices are minimum, the diminution of the average number of circulating erythrocytes being of 2.46% (EPfHr) and 6.91% (EPfRc) respectively, the decrease of the hematocrit of 2.45% and 5.91% respectively, as well as the reduction of erythrocytes hemoglobin amount of 9.04% and 11.62% respectively;
- a slight growth of the white blood cells total number (16.7% and 12.4%), in the case of the EPzAgsp and EPfRC, while the EPfHr polyphenolic bioproduct causes an nonsignificant decrease, of only 1%, of the white blood cells;
- a significant increase in the average number of circulating platelets (64.5%) consequent to the polysaccharidic treatment, as well as a decrease of the average number of thrombocytes after the polyphenolic treatment, the maximum of the negative impact being in the case of the polyphenolic biopreparation extracted from *Hippophae rhamnoides* (34.2%).

The reported haematological variations suggest a minor toxic impact of the studied extracts upon the functionality of the reticulo-histocitary system and on the viability of the circulating blood elements.

Another experimental aspect, approached during our investigations, was the monitoring of the impact of the new natural polysaccharidic and polyphenolic extracts upon some plasmatic biochemical parameters of the animals bearing Walker 256 carcinosarcoma. In this respect, we followed the quantitative modifications of the plasma total proteins, albumins, globulins, glycemia, lactacidemia, lipemia, cholesterolemia and free fatty acids of the animals treated with the polysaccharidic or polyphenolic agents. Our option is related to the fact that any significant influence, whether positive or negative, upon these indices could indicate the presence or absence of a toxic effect upon the cell intermediary metabolism of the studied biopreparations. The obtained results were procentually expressed and are graphically presented in Figure 2.

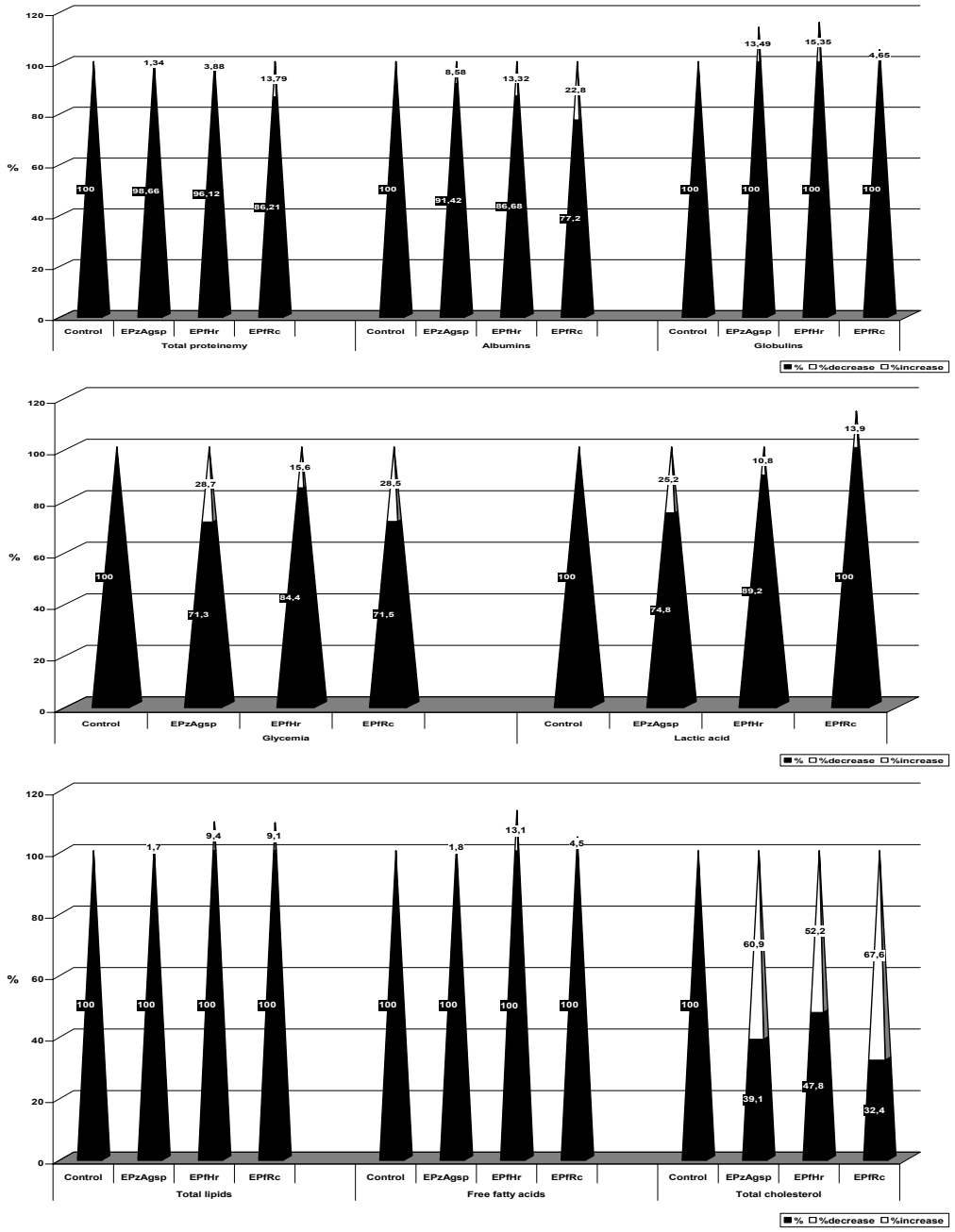


Fig. 2 The sense and amplitude of the plasmatic variations of the proteinemy, albuminemy, globulinemy, glycemy, lactacidemy, lipemy, cholesterolemy and the free fatty acids, registered after the treatment with EPzAgsp, EPfRc şi EPfHr products.

The daily intraperitoneal treatment of the tumor-bearing animals with the polysaccharidic or the two polyphenolic extracts has induced, as compared to the control group:

– minor reductions of the plasmatic proteinemy (with percentage values ranging between 1.0% and 14.0%) and albuminemy (with percentage values ranging between 8.6% and 22.8%), correlated to an important increase in the plasma globulins level (of approximately 15.4%), which suggests a possible immunostimulatory impact of the studied extracts;

– moderate hypoglycemias (of 28.7% for EPzAgsp treatment, 15.6% for EPfHr and 28.5% for EpfRc), hypolactacidemias (of 25.2% for EPzAgsp, 10.5% for EPfHr) and hyperlactacidemia (of 13 9% for EPfRc);

– slight increases of the plasma total lipids (the recorded highest percentage value was 9.4%) and of the free fatty acids (the maximum reached being of 13.1%), exception making the polyphenolic biopreparation obtained from *Rosa canina*, which causes a reduction of the free fatty acids level of only 4.5%;

– significant decreases of the plasma total cholesterol concentration, the highest level being registered in the case of the EPfRc treatment (67.6%). Thus, it can be suggested a hypocholesterolemiant impact of the studied agents.

The qualitative and quantitative assessment of the antitumoral pharmacodynamic action of a new chemical agent is not sufficient for its characterization as potential oncochemotherapeutic drug, because it is also necessary and the evaluation of the toxicity of the new candidate as to enrich the fighting arsenal against malignant disease, in order to establish the superiority of its benefic features toward the toxic ones. Since the treatment with cytostatics, most often, involves the use of high dosages – uniquely able to determine an efficient antineoplastic impact - there is the risk of injuring the normal cells from the cancer aggressed organism, therefore of inducing some dangerous toxic side effects, which diminish the chemotherapy importance.

Assessing the toxicity degree, an essential condition for the appreciation of the therapeutic significance of a proven oncostatic agent, involves, among others: the analysis of some haematological parameters behavior, their modification from the normal limits suggesting different degrees of toxicity upon the reticulo-histiocitary system; the establishment of the quantitative and qualitative profile of some plasmatic biochemical indices, its disturbance suggesting more or less profound perturbations of the cell metabolism at the animals subjected to experimental treatment with the studied agent (Cotrau, 1978; Mogos and Sitcai, 1988; Ninomiya et al, 2005; Valeriote et al., 1984; Voicu, 1997).

This is the experimental background in which our research has been framed, aiming at the evaluation of the EPzAgsp polysaccharidic or EPfHr and EPfRc polyphenolic biopreparations impact upon the normal development of the different physiological processes of the trial animals with neoplasm. These data are necessary for fulfilling the preclinical characterization of the polysaccharidic or polyphenolic extracts as possible antineoplastic agents., from a toxicological point of view,

The appreciation of the results, obtained during the evaluation of our natural agents toxicological impact upon the animals aggressed by the experimental neoplasm, requires their analysis in accordance to the stipulations of the reference screening programs, imposed for this preclinical investigation stage. Thus, the World Health Organization (WHO) established a 5 levels scale for the appreciation of the hematological toxicity of an anticancer treatment: *level 0* includes the cytostatics which do not affect the normal values of the leukocytes, erytrocytes, the hemoglobin and the hematocrite; *level 1* comprises the oncochemotherapeutics which modify by

approximately 13,8% the number of circulating leukocytes or by 7% the mean number of erythrocytes, the hematocrit and the hemoglobin; *level 2* includes the cytostatics that determine a modification in the leukocytes number of 38,8% or of the erythrocytes, hematocrit and hemoglobin of 21%; *level 3* comprises the drugs which modify by 63,2% the number of circulating leukocytes or by 35% the mean number of erythrocytes, the hematocrit and the hemoglobin; *level 4* includes the cytostatics that determine a modification in the leukocytes number of 75-77.5% or of the erythrocytes, hematocrit and hemoglobin of 41% .

One of the most vulnerable tissues to the cytotoxicity of the xenobiotic agents of different nature is the reticulohistiocitary one, tissue which represents the basic structural element of the hemato-, leuko-and thrombopoietic organs, of the reservoirs and "cemeteries" of the figurate blood elements. Therefore, tracking the effects of some biologically active agents upon the haematological parameters is an experimental model suitable for the preliminary assessment of their toxicity on the normal cells from the structure of the erythro-, and leukopoiesis organs – erythro- and leucolytic organs of the healthy or tumor-bearing animals.

Therefore, recording a minor reduction (of 17.9% in the case of the polysaccharidic agent, and of 2.5% and 6.9% in the case of the polyphenolic extracts) of the total number of circulating red blood cells, a negligible decrease in the hemoglobin load of the red cells (with 17.9% for EPzAgsp, 2.4% for EPfRc and 5.9% for EPfHr), an nonsignificant reduction of the hematocrit (with a maximum value of 22.8%), as well as a moderate increase or decrease in the average number of circulating blood white cells and platelets, reveals a moderate toxic impact of the fungal or vegetable extracts both upon the blood figurate elements and their formation organs . Moreover, the analysis of the recorded results, in this experimental framework, through the values established by WHO for assessing the haematological toxicity of a medicinal agent, allows us the insertion of the polysaccharidic and polyphenolic extracts in Group 1 of haematological and leukocitary toxicity, which includes medicines that determine changes of about 7% of the average number of circulating erytrocytes, of hemoglobin and hematocrit, as well as of about 13.8% of the average number of circulating leukocytes(Gossel and Douglas, 1994; Voicu, 1997).

A change in the homeostasis of the internal environment after the penetration of a xenobiotic agent in the organism – depending on the sense and duration of the induced disturbance – gives information on the toxicity of a drug. In this regard, we proposed that, in the preliminary assessment of the toxicity of the polysaccharidic or polyphenolic agents, to also take into account its effect upon certain biochemical blood constants at rats bearing the Walker 256 carcinosarcoma. Following this path, the moderate quantitative changes of various plasma biochemical constants - hypoproteinemy, associated with plasmatic hypoalbuminemia and hyperglobulinemia, slight hypoglycemia, accompanied by hypo- or hyperlactacidemy, minor hyperlipemy correlated to a plasmatic hypocholesterolemia (!!!in textul in romana le-ai pus pe toate la plural, vezi tu care trebuie) and with moderate increased or decreased levels of the plasmatic free fatty acids, suggest a low cellular alteration degree of the protidic, carbohydrate and lipid intermediary metabolism processes, with importance on the quality of animal life, being given the structural, functional and energetic role of these biochemical constituents.

The bulk of our experimental results allows us to appreciate a moderate toxic impact of the polysaccharidic and polyphenolic biopreparations upon the normal cells of the animal organism agressed by the experimental neoplasm and, particularly, upon the organs directly involved in maintaining the homeostasis of the internal environment.

CONCLUSIONS

The reduced toxicity of the polysaccharidic and polyphenolic experimental treatment is compatible with the normal development of the physiological processes in the animal organism aggressed by neoplasm.

Three new pharmacodynamic effects of the fungal and vegetable polysaccharidic and polyphenolic biopreparations are outlined, these seeming to act as immunomodulatory, normolipemiant and hypocholesterolemiant agents.

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