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MINIMAL RESIDUAL DISEASE (MRD) IN LEUKEMIA

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Abstract: Resistance to therapeutic agents is a main factor in the failure of cancer treatments. In leukemia, the resistant cells remaining in the bone marrow or peripheral blood constitute minimal residual disease and can be detected by highly sensitive assays when the patient appears to be in complete remission. Currently three different techniques with sensitivity of at least 10^{-3} (one leukemic cell between 10^3 normal cells) are used for MRD detection: flow cytometric immunophenotyping, which is based on the detection of abnormal or unusual phenotypes; PCR analysis of patient-specific junctional regions or rearranged immunoglobulin (Ig) or T cell receptor (TCR) genes and PCR analysis of break-point fusion regions of chromosomal aberrations. Studies of MRD provide means of detecting relapse at sub-clinical levels and permit early intervention, this method being, therefore, highly useful in improving the oncology patient's clinical management. This paper aims to present, from a large body of data among the patients with leukemia, the current state and development of molecular techniques in the growing field of emerging methods that can detect MRD.

INTRODUCTION

Acute leukemia includes a heterogeneous group of neoplastic disorders with great variability in clinical course and response to therapy, as well as in the genetic and molecular basis of the pathology. In these types of aggressive cancers, the bone marrow makes a large number of abnormal white blood cells that are not fully developed.

Minimal or submicroscopic residual disease (MRD) represents a small number of leukemic cells that remain in the patient's body during treatment or after treatment when the patient is in remission. This is the major cause of relapse in cancer and especially leukemia, thus studies during treatment hold great potential for improving the clinical management of patients with acute leukemia.

Cancer cells are the result of a single malignantly transformed cell, therefore these cells are clonally related. Hence, monoclonality is a key feature of malignant tumor cell populations, which allows discrimination between oligo or monoclonal and polyclonal reactive processes (Langerak et al, 2007). Clonality assessment and detection is possible via several technical approaches such as the study of chromosomes, DNA markers, tumor specific proteins and patterns of proteins known as tumor phenotypes.

One of the many different markers that can be used for clonality testing in suspected lymphoproliferations is the immunoglobulin (Ig) and T-cell receptor (TCR) antigen receptor gene rearrangements (Langerak et al, 2012). These Ig and TCR rearrangements occur in developing lymphocytes during the early stages of T and B cell maturation through somatic V(D)J (V-variable, D-diversity and J-joining) recombination and results in the highly various repertoire of antibodies/immunoglobulins (Igs) and T cell receptors (TCRs) found in B cells and T cells, respectively (Owen et al, 2013).

The most widely currently applied MRD assays in acute leukemia are flow cytometric identification of aberrant immunophenotypes and polymerase chain reaction (PCR) amplification of fusion transcripts and rearranged antigen receptor genes (Neale et al, 2004).

MRD assays can accurately measure treatment response and allow estimates of the residual leukemic cell load during clinical remission in individual patients, thus improving the selection of therapeutic strategies and, potentially, long-term clinical outcome.

CYTOGENETIC AND MOLECULAR ANALYSIS OF MINIMAL RESIDUAL DISEASE IN LEUKEMIA

In the last decade the rapid progress in understanding the etiology of leukemic malignancies and technological advances has increased the specificity and sensitivity of detection of cancer cells at patients who appeared to be cured or in remission by conventional techniques (Cross 1997). Therefore, the therapeutic response of the patient can now be assessed by monitoring minimal residual disease (MRD) which means the detection of malignant cells at $\geq 1 \times 10^{-4}$ sensitivity, at subclinical levels.

For a long period of time, clinicians relied on examination of cellular morphology in peripheral blood and marrow collected at regular intervals from asymptomatic patients in order to observe the patient's long-term response to therapy. These studies were and remain important but they lack sensitivity, as a consequence, the patients frequently had advanced disease that was difficult to treat by the time that relaps was detected (Kaeda et al, 2002).

Cytogenetic techniques

By means of morphological techniques in leukemias and non-Hodgkin lymphomas the detection limit for the identification of reduced number of malignant cells is not lower than 1% (1 malignant cell among 100 normal cells). Therefore, van Dongen et al (1986) proposed different techniques for the detection of small numbers of cancer cells such as cytogenetics, cell culture systems, premature chromosome condensation and recombinant DNA techniques. Unfortunately, most of these techniques do not lower the 1% detection limit.

Acute leukemia is generally considered to be in remission when cancer cells reach less than 5% of the bone marrow cell population; however, Ryan and van Dongen (1988) demonstrated that patients with acute leukemia may have approximately 10^{12} malignant cells at diagnosis and those in remission by this criterion may have as much as 10^{10} undetectable neoplastic cells.

Conventional karyotyping based on chromosomal abnormalities that were observed at diagnosis has been used to monitor residual disease. The main advantage of this method is that it permits clear identification of the leukemic cells. In early studies of patients with acute myeloid leukemia (AML) the disappearance of the abnormal karyotype is generally correlated with the clinical remission as Testa et al (1979) observed in a study of patients with acute myeloid leukemia. They did not detect abnormal cells in patients in remission, although single abnormal cells of clonal origin could occasionally be observed. Hart et al (1971) reported the same observation in a study involving 10 patients with chronic myeloid leukemia (CML); each of the patients who relapsed had the same karyotypic abnormality that was found at diagnosis. More recently, Freireich et al (1992) reported that abnormal metaphases, also identical to those found at diagnosis, were observed at 20 of 71 patients with acute myeloid leukemia in morphologic remission. All 20 patients relapsed in the next period of time, thus maintaining the reliability of positive karyotypic analysis as a predictor of eventual relapse. Nevertheless, a number of 25 patients within the 51 patients with negative findings also relapsed, indicating that failure to detect abnormal clones does not necessary guarantee durable remissions. Therefore, karyotypic changes can have prognostic significance which may be useful in decisions on the aggressiveness of further therapy.

Metaphase analysis by conventional banding techniques is a laborious procedure with a success rate depending on the number of metaphases that can be observed and on the proliferative rate of leukemic cells that varies from case to case. Thus, there has been considerable effort to develop techniques that would ease metaphase screening.

Fluorescence in situ hybridization (FISH) techniques are based on chromosome-specific and gene specific DNA probes to identify numeric and structural chromosomal abnormalities. FISH can be combined with morphologic analysis to amend the accuracy of the results (Pinkel et al, 1988). The main advantage of this technique is that it provides interpretable information with use of nondividing cells, raising the chances of identifying abnormalities among the cells having a reduced proliferative rate.

The sensitivity of FISH technique was well demonstrated by research of Heerma et al (1993) who used probes of chromosomes X, 10, 17 and 18 to observe early remission in patients with acute lymphoid leukemia (ALL). The mean number of aneuploid cells observed in three normal

bone marrow samples was 8 of 2000, and one month after diagnosis, tri an tetrasomic interphases increased significantly compared to control values in three of the seven patients studied; also pentasomy and hexasomy that were not found in control samples were observed in five of the seven cases studied.

Nylund et al (1994) used FISH to detect numeric chromosomal abnormalities in interphase and metaphase cells and targeted translocations in metaphases cells in patients with various hematologic malignancies. From seven patients with AML, three had cells with abnormal karyotype in morphologic remission bone marrow samples, two of whom relapsed. By contrast, none of the FISH-negative remission samples patients had a recurrence. The conclusion was that FISH analysis of remission in ALL was less informative and although no abnormal karyotypes were detected in remission bone marrow samples of five patients, two relapsed.

The limitation to the presence of aneuploid but not leukemic cells makes the sensitivity of MRD analysis by FISH approaches only 1% (Gray et al, 1990). Methods to detect structural chromosomal abnormalities in interphase cells by FISH with locus-specific probes have also been developed. A well-known example is *BCR* and *ABL* genes labeled with different fluorochromes used to identify the t (9;22) translocations.

In the 1980s, main approach to assess the response to treatment was repeated bone marrow metaphases analysis for the presence of the Philadelphia (Ph) chromosome in patients. Bartram et al (1983) described initially the application of in situ hybridization for detection of the translocation of *ABL* to the Ph chromosome. In around 90% of cases of chronic myeloid leukemia, chromosomal material is reciprocally exchanged between the long arm of one chromosome 9 and the long arm of chromosome 22, translocation referred to as t(9;22)(q34;q11). The derivative 22q- is the Ph chromosome. The hallmark of chronic myeloid leukemia (CML) is the formation of a *BCR-ABL* fusion gene, usually as a result of the Ph chromosome translocation. Melo et al (1993) showed that the *ABL-BCR* gene formed on the derivative chromosome 9q+ is transcriptionally active in 65% of the CML patients involved in their study. However, the sensitivity of this approach may have limitations because in about 5% of normal lymphocytes artifactual colocalization may appear (Arnoldus et al, 1990).

Flow-cytometry

Some groups have proposed the use of flow-cytometry as mean to detect chromosomal abnormalities, having a higher accuracy than conventional banding techniques. The first approach to investigate the applicability of this technique was done by Arkesteijin et al, in which they used a two-colour analysis with chromomycin A3 (which labels GC base pairs) and Hoechst 33258 (which labels AT base pairs), procedure that resolved all chromosomes except numbers 9 to 12. The analysis revealed the percentage of subclones containing a certain chromosome anomaly, confirmed by the conventional cytogenetic analysis. Although it is not possible by this technique to determine the position of the breakpoint, the involved chromosomes in the translocation event could be identified, but also, in some cases, the low percentages of aberrations could not be detected. This study showed that CML can be diagnosed on the basis of flow karyotypic results and additional chromosomal aberrations can be detected provided that changes in the amount of DNA per chromosome have occurred. The precise quantification of the composition of subclones in the case of mosaicism appears difficult.

Another method of monitoring MRD relies on the identification of aneuploidy by single-laser cytometry in cells labeled with DNA-binding fluorochromes such as propidium iodide and 7-actinomycin D (Rabinovitch et al, 1986). Pantazis et al (1987) observed the course of a patient with

AML reporting that the disappearance of aneuploid peaks in flow cytometry coincided with morphologic remission and Redner et al (1990) detected early relapse in a patient with acute lymphoid leukemia that was in clinical remission.

Nevertheless, flow-cytometry has some specific limitations. Extreme sensitivity, such as detection of 1 leukemic cell among 10^5 or more normal cells is difficult to achieve by flow-cytometry and such high sensitivity is important in studies deeking MRD in patients. Another limitation is that the immunophenotype of leukemic cells may change during the progression of the disease, which may conduct to a false-negative result (Baer et al, 2001).

Molecular studies

Though cytogenetic analysis and FISH studies remain extremely valuable in the initial investigation of malignant hematopoietic disorders, their role in monitoring MRD has decreased with the introduction of molecular techniques.

The PCR technique allows the amplification of tumor-specific DNA sequences or mRNA sequences (after reverse transcription into cDNA), if the flanking sequences are well defined. This PCR-mediated amplification can detect specific sequences derived from only a few cancer cells among normal cells. Well-defined chromosome translocations such as t(9;22) have been used as tumor-specific markers (van Dongen et al, 1991). The advantage of using specific chromosome aberrations as tumor-specific markers is their stability during the progress of the disease. Whether genomic DNA or cDNA obtained from RNA is used in this procedure depends on the molecular target, this method having an extremely high sensitivity for detecting kariotypic abnormalities. Experiments with artificial mixtures of leukemic and normal cells were conducted by Cross et al (1993) and have consistently shown detection of a single leukemic cell among 10^5 to 10^6 cells.

Among the leukemic lymphoblast-specific fusion transcripts that lately became targets of PCR analysis, only those connected with the Ph chromosome have been repeatedly applied in the study of MRD (Miyamura et al, 1992). Miller et al. (1993) showed that serial negative results obtained using PCR were correlated with prolonged disease-free survival of the patient, whereas one or more positive tests after treatment were associated with subsequent relapse.

The RT-PCR has been widely exploited to detect the different *BCR-ABL* transcripts by multiplex PCR (which has a sensitivity of 10^{-2} – 10^{-3}) and has enhanced the level of sensitivity of MRD detection. A sensistivity of 10^{-5} – 10^{-6} is achievable by nested PCR in a clinical laboratory (Biernaux et al, 1995); in these conditions *BCL-ABL* mRNA was detected in a high proportion of normal healthy individuals (Bose et al, 1998). A sensitivity of 1×10^{-5} is achievable by Q-PCR, but a major concern is contamination and false-negative results due to the lack of mRNA or sub-optimum integrity of mRNA/cDNA (Beillard et al, 2003).

DETECTION OF ANTIGEN-RECEPTOR GENE REARRANGEMENTS

The antigen-receptor genes include several discontinuous germline segments (V-variable, D-diversity and J-joining) that undergo clonal rearrangements in lymphoid cells. Analysis of Ig and T-cell receptor (TCR) gene configurations can be used to monitor the persistence of malignant clones whose rearrangements were determined at diagnosis. The B and T-clonal recombinations generate patient-specific DNA length and sequences which are ideal molecular markers for detection and quantification of leukemic cells among normal lymphocytes in remission samples. Although sensitive, this technology is susceptible to false-negative results due to clonal evolution during the progression of the disease, thus some patients may relapse with a clone different to the

one observed at diagnosis. The risk of false-negatives can be diminished by targeting two Ig/TCR gene rearrangements when conducting MRD-PCR studies (Van der velden et al, 2008).

The sensitivity of PCR for antigen-receptor gene rearrangements varies with the uniqueness of the leukemia-specific regions of the genes (Bregni et al, 1989). In a study conducted by Brisco et al (1994) PCR amplification of IgH genes followed by hybridization with clonospecific probes lead to a detection level of 1 leukemic cell in 10^4 or fewer normal cells in 42 of 88 cases. Similarly, in 71 cases studied by Bartram et al (1993) with PCR amplification of TCR genes, the detection level reached 1 leukemic cell in 10^4 or fewer normal cells in 33 cases; a sensitivity of 1 in 10^5 was achieved in 29 cases, whereas 1 in 10^6 was obtained in only 9 of the 71 cases.

Specific primers for individual V and J regions or consensus primers for conserved regions can be designed. For example, the approximately 100 VH genes can be grouped into seven different families with homologous sequences (Stewart et al, 1994). The most conserved regions known as framework regions and the regions that encode the antigen-binding site of the Ig heavy chains are also known as complementarity-determining regions (CDRs). The CDRs that are encoded by the VH gene region are CDR1 and CDR2; by contrast, the CDR3 region comprises in the 3' end of VH, all of D and 5' end of JH, and the N nucleotides assembled during the recombination process. This region is specific to each lymphoid clone. To amplify the rearranged IgH genes, a consensus JH primer, a panel of VH primers specific to VH families can be used (Deane et al, 1990), thus detecting the Ig gene rearrangements in 90% of the cases of B-lineage ALL cases at diagnosis as Deane et al, reported with a 75% success rate.

In T-lineage ALL the issue that appears is that IgH rearrangements are usually incomplete. TCR α and TCR β genes have a large number of functional V and J segments whereas TCR γ and TCR δ contain only a few (Davis et al, 1988), thus the potential for combinatorial diversity is higher. Efforts to determine rearranged TCR γ genes using consensus primers were successful in approximately 90% of the cases (D'Auriol et al, 1989).

Nevertheless, in contrast with abnormal gene configurations caused by chromosomal translocations, the detection of a PCR-amplified signal from antigen-receptor genes cannot be taken as evidence of MRD until the signal is differentially distinguished from the background, originating from normal lymphoid cells. This can be resolved by amplifying the clonal IgH rearrangements with single VH family-specific primers, then identifying them on the basis of size and signal intensity after separation by high-resolution gel electrophoresis (Cole-Sinclair et al, 1993). The electrophoretic profiles obtained with remission samples are compared with the diagnostic DNA for the presence of similar dominant bands. Clonally rearranged Ig and TCR genes can be observed by analyzing the junctional regions TCR γ or TCR δ genes or the CDR3 regions of the IgH genes. Similar to PCR amplification of translocation breakpoints, the leukemia-specific primers detect PCR-amplified signals only in the presence of the malignant clone. Beishuizen et al (1994) detected bi or oligoclonal IgH rearrangements at diagnosis in 8 of 30 cases of B-lineage ALL and also noted differences in the rearrangement of patterns in 20 of the cases at the time of relapse. They observed a correlation between shifts in antigen-receptor gene rearrangement pattern and the duration of remission, although in 75% of the cases at least one major rearranged IgH, TCR γ or TCR δ allele remained the same at relapse. Clonal evolution and oligoclonality in IgH genes are caused by disruption in the V-N-D portion, whereas D-N-J sequences are left unmodified. Davis et al (1991) analyzed the IgH gene by PCR at diagnosis and relapse in 12 cases of ALL and detected clonal evolution in 4 cases attributed to VH gene replacement and in 3 cases due to new rearrangements with loss original alteration. Stewart et al, identified changes in the pattern of PCR amplification between diagnosis and relapse in 12 of 39 patients with B-lineage ALL studied for

IgH gene rearrangements. In 9 of 12 cases, there were observed subclones or rearrangements of partial or complete configurations determined at diagnosis. However, major pitfalls of this application are the occurrence of multiple rearrangements at diagnosis (oligoclonality) and modified patterns that appear at relapse (clonal evolution), which may lead to false negative results of MRD-PCR technique (Van Dongen et al, 1991).

PCR based clonality testing in lymphoproliferations combined with immunohistology and with results from flow-cytometric immunophenotyping offer an integration of all available data to reach the most reliable diagnosis.

CONCLUSIONS

Accurate determination of MRD had a profound impact in the clinical management practices of patients with hematologic malignancies. Prospective studies in large series of patients have demonstrated a strong correlation between MRD levels during clinical remission and treatment outcome. Therefore, MRD assays can be used to assess early response to treatment and predict relapse. To conclude, there is a need to integrate the molecular data with data from immunohistology and also flow-cytometric immunophenotyping for an appropriate interpretation and treatment effectiveness.

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MECHANISM OF ACTION OF SOME NEW CYTOSTATIC/CYTOTOXIC POLYPHENOLIC EXTRACTS FROM *VITIS VINIFERA* SEEDS

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Abstract: *Vitis vinifera* seeds are very rich in bioactive compounds, especially polyphenols, representing a generous and available source for obtaining of new active biopreparations with agricultural, biomedical and ecological valorification. In this paper are presented the results focused on identification of the possible action mechanisms of some total polyphenolic bioproducts, extracted from grape seeds (P4, P5 in doses of 200 and 300 µg/mL) and proven already in our previous tests as cytostatic/cytotoxic agents. For this purpose were analyzed, on tumoral HeLa and normal Vero cells, the cell apoptosis process (Annexin V-PI assay) as well as the levels of ROS (DCFH-DA assay). It was registered, after the polyphenolic treatment period (48 hours), a stimulation of the apoptosis in HeLa cell cultures, the maximum effect being in the case of the bioproduct coded P4, at a dose of 300 µg/mL. In Vero cell cultures, the intensity of apoptosis was similar to the control group. No variations in ROS levels, as compared with control group, were registered in both cell cultures. Therefore, the cytotoxic effect of the studied polyphenolic extracts seems to be induced by activation of the cell apoptosis process and not by oxidative mechanism.

INTRODUCTION

In the last decades, the cancerous disease has become one of the most frequent cause of the human mortality, its presence being very difficult and insecure to diagnosis in his early stages. Despite the fact that the understanding of the mechanisms underlying the initiation, progression and spreading of the cancer in the organisms has registered important progressions, its treatment with nowadays methods is still ineffective in many cases. So, the actual chemotherapeutic research is oriented to the identification of new sources for antineoplastic compounds, with a more pronounced selectivity for cancerous cells, paying a special attention to the natural resources.

Wastes from wine industry are representing a large source of possible new bioactive products, useful in the organic agriculture, in animal farming or even in human therapy. Development of a highthroughput technology able to release, identify and concentrate the biological active compounds from wine waste could respond, among other things, at two important problems: identification and validation of new antineoplastic compounds and waste depletion.

Currently, there are several developments regarding winery waste valorification that should be taken into account to select the best available technique for the winery waste recovery and recycling by different methods as presented in table 1 (Oliveira & Duarte, 2016)

Table 1. Treatment of different solid waste from wine industry and their possible use (after Oliveira & Duarte, 2016)

	Treatment	Use
grape marc	fractionation of grape seed	polyphenol production
	hydrolysis and fermentation	lactic acid production
	hydrolysis and fermentation	biosurfactants and bioemulsifiers production
	distillation	ethanol and tartaric acid production
	extraction	tannins, polyphenols and oil production
	fermentation of grape seed	laccase production
lees	composting	plant substrate
	solubilization and precipitation	tartaric acid production
	composting	plant substrate

stalks	composting	plant substrate
	lyophilisation and extraction	polyphenol production
sludge	co-composting	plant substrate
	anaerobic digestion	biogas production

By applying different treatment methods, the seed waste from the wine industry has recently become a natural resource useful in obtaining of some bioproducts with practical capitalization as fertilizer, antimicrobial, antifungal, cytostatic, healing, immunomodulating, antioxidant agents, etc. Beside the benefits of obtaining new bioactive compounds, the environmental cleaning impact of the wastes is also an important objective acquired.

The polyphenolic substances are secondary metabolites abundant in different vegetables, fruits, cereals, including and dimers, oligomers and polymers of catechins (monomers of flavan-3-ols, the most chemically complex subclass of flavonoids) (Fantini et al., 2015).

The broad pharmacological spectrum and medicinal properties of the polyphenols from grape seed include benefits against the cardiovascular dysfunctions, acute and chronic stress, gastrointestinal distress, neurological disorders, pancreatitis, various stages of neoplastic processes (carcinogenesis). A significant cytotoxicity towards human breast, lung and gastric adenocarcinoma cells in parallel with the improvement of the growth and viability of normal cells was noticed (Bagchi, Bagchi & Stohs, 2002; Bagchi, Swaroop, Preuss, & Bagchi, 2014). Also, these chemical compounds are agents useful in the detoxification of carcinogenic metabolites, in the scavenging of free radicals, released as a result of oxidative stress. The antioxidant ability is significantly better than of vitamins C, E and beta-carotene oxidative stress scavengers.

Therefore, *Vitis vinifera* grapes represent a generous and available source for obtaining new actively biopreparations with agricultural, biomedical and ecological capitalization. In this paper are presented our results focused on the identification of the possible action mechanisms of some new total polyphenolic bioproducts, extracted from grape seeds (P4, P5 in doses of 200 and 300 µg/mL) and proven already in our previous tests as cytotoxic agents, like other polyphenolic preparations obtained in the past (Savin et al, 2009; Nechita et al., 2011).

Thus, the *in vitro* experimental approach was oriented to the cell apoptosis identification and oxidative stress levels assessment in normal and neoplastic cells treated with polyphenolic extracts, obtained from grape waste, in order to identify and understand their mechanism of action.

MATERIALS AND METHODS

The polyphenolic biopreparations were obtained from *Vitis vinifera* seeds - from grape marc after the oil removal by cold pressing - by several extraction methods with: supercritical fluids (liquid CO₂ and 98% ethanol, P1); water, at 15 bar (P2) or 3 bar (P3); **78 % ethanol** at 15 bar (P4) and 3 bar (P5) pressures.

Neoplastic HeLa and normal Vero cells were seeded in DMEM medium supplemented with fetal bovine serum (FBS) in 24 well plates at a density of 50.000 cells / well. After 24 hours from cell cultures initiation, the growth medium was replaced with fresh complete medium containing P4 and P5 compounds, in doses of 200 and 300 µg/mL. After 48 hours from adding the compounds, the levels of reactive oxygen species (ROS, by DCFH-DA assay) as well as of apoptosis processes (by Annexin V-FITC assay) were investigated.

DCFH-DA assay. The cells (both from control and treated groups) were harvested by trypsinization, washed twice with PBS and finally resuspended in cold PBS. The cells were stained with 5 µL of 5 mM DCFH-DA solution and incubated 30 minutes at 37°C. Before flow cytometer analysis, to the cell suspension was added 0.5 µL of propidium iodide solution (1 mg/mL). The experimental model consisted in the discrimination between alive and dead cells and the determination of ROS levels only in the alive cells.

Apoptosis assay. After treatment, the cultures were trypsinized, the cells being washed with cold PBS, resuspended in binding buffer and successively marked with Annexin V-FITC and propidium iodide.

The registration of ROS and apoptosis levels was performed with a Beckman Coulter Cell Lab QuantaSC flow cytometer, equipped with a 488 nm laser and with specific excitation and collection filters suitable for the selected fluorochromes.

The collected data were exported as LMD files and analyzed with Flowing Software (developed by Perttu Terho, Turku University, Finland).

All of the experiments were carried out with at least three independent repetitions and all data were expressed as the mean value and standard error of mean (SEM). The statistical analysis was performed using Student's "t" test and the differences were expressed as significant at the level of $p < 0.05$ [Cann, 2002].

RESULTS AND DISCUSSIONS

The dynamic levels of the reactive oxygen species, produced by the metabolic reactions, are permanently maintained in equilibrium by different mechanisms, playing important roles in the cellular metabolism and cell signaling. The effect of different reactive species is dependent by their specific concentrations. Thus, it can be either positive, modulating different signaling networks, or negative, influencing DNA integrity or lipid peroxidation.

Table 2. Percentage distribution of ROS negative and positive cells after incubation with different doses of the P4 and P5 products.

Group	Vero cells				HeLa cells			
	ROS -		ROS +		ROS -		ROS +	
	X±SEM	p<	X±SEM	p<	X±SEM	p<	X±SEM	p<
Control	99.92±0.05	-	0.08±0.05	-	99.95±0.02	-	0.05±0.02	-
H ₂ O ₂ 5 mM	83.50±1.14	0.001	16.50±1.1	0.001	82.42±1.50	0.001	17.58±1.50	0.001
P4 200 µg/mL	99.94±0.02	NS	0.06±0.02	NS	99.96±0.01	NS	0.04±0.01	NS
P4 300 µg/mL	99.96±0.02	NS	0.04±0.02	NS	100.00±0.00	<0.05	0.00±0.00	<0.05
P5 200 µg/mL	99.20±0.33	0.05	0.80±0.33	NS	99.98±0.01	NS	0.02±0.01	NS
P5 300 µg/mL	99.25±0.24	0.05	0.75±0.24	<0.05	100.00±0.00	NS	0.00±0.00	NS

Prolonged period of incubation (48 hours) of normal and cancerous cells with P4 and P5 didn't significantly influenced the intracellular levels of ROS, those being similar to the control group, as it can be noticed from above table (table 2). In the positive control, in which cells were treated with a solution of 5 mM H₂O₂ within 20 minutes before readings, the production of ROS was intensified with about 17% in both cell lines.

Apoptosis is a dynamic process, essential for the normal development of the multi- and pluricellular organisms, being strictly controlled by different cellular mechanisms, maintaining the normal cellular homeostasis (Elmore, 2007) (Häcker, 2000) (Danial & Korsmeyer, 2004) (Moquin & Chan, 2010).

Investigation of apoptosis by annexin V / propidium iodide resides in the strong affinity of annexin V for phosphatidylserine residues (normally hidden within the plasma membrane) on the surface of the cell. During apoptosis, phosphatidylserine is translocated from the cytoplasmic face of the plasma membrane to the cell surface. Propidium iodide is used to discriminate between dead and alive cells, allowing also separation between preapoptotic and apoptotic cells in combination with Annexin V.

The apoptotic intensity in tumoral HeLa and normal Vero cells was assayed after 48 hours of incubation with different doses of the P4 and P5 polyphenolic extracts, the experimental results being included in the figures 1 and 2.

In HeLa cells, the frequency of preapoptotic and apoptotic cells was significantly increased, above the specific frequency of the control group. Due to increase of the apoptosis intensity, the frequency of the dead cells has also augmented in the HeLa cell cultures.

In the case of the P4 biopreparation, the increase of dose determined the amplification of the preapoptotic and cytotoxic effects, as shown in the figure 1A. The P5 compound didn't achieved a higher preapoptotic or cytotoxic effect as related to the increase of the dose, the values between

both doses being similar (figure 1B).

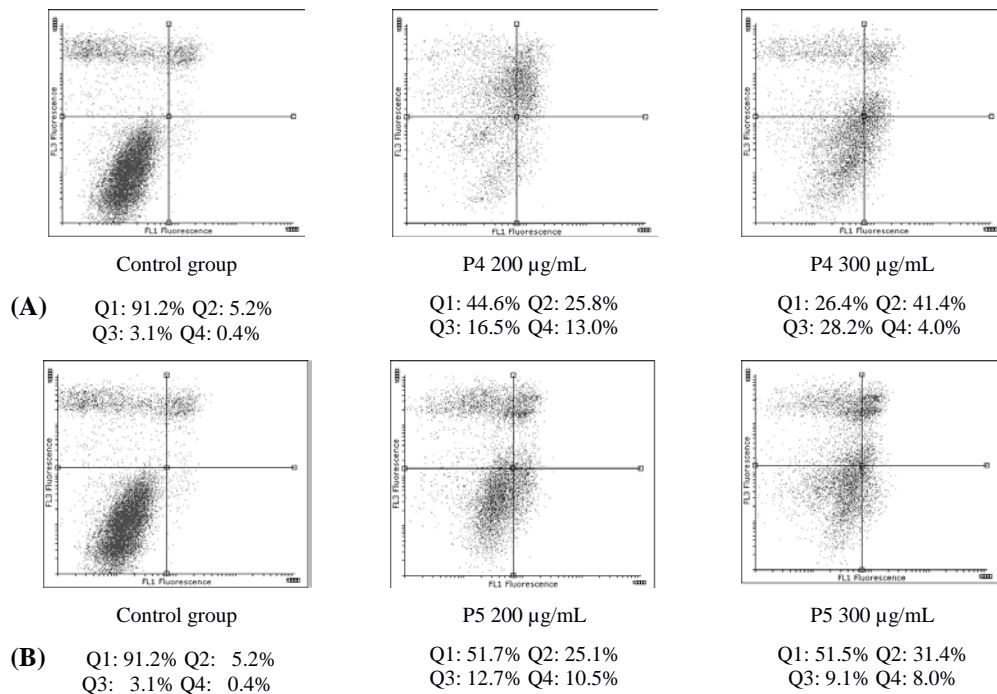
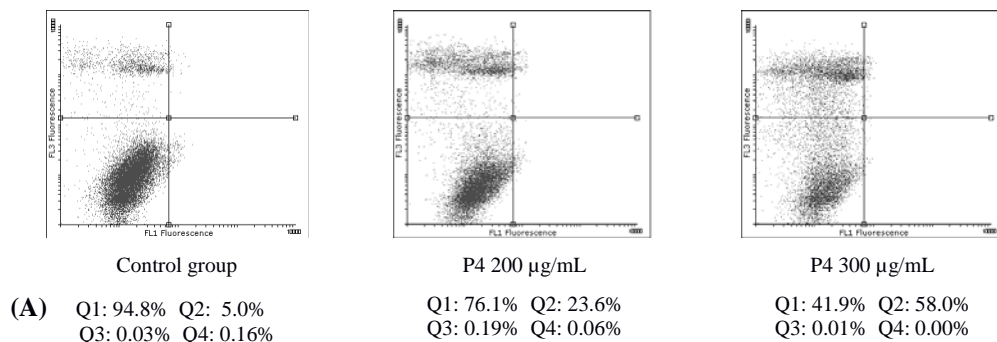


Figure 1. Cytograms of bivariate analysis (Ann V-Fitc vs PI) for cell apoptosis and viability identification in HeLa cultures treated with different doses of P4 (A) and P5 (B) polyphenolic extracts. Q1 (left-down quarter) denotes viable cells , Q2 (left-up quarter) – dead cells , Q3 (right-up quarter) – apoptotic cells and Q4 (right-down quarter) – preapoptotic cells.



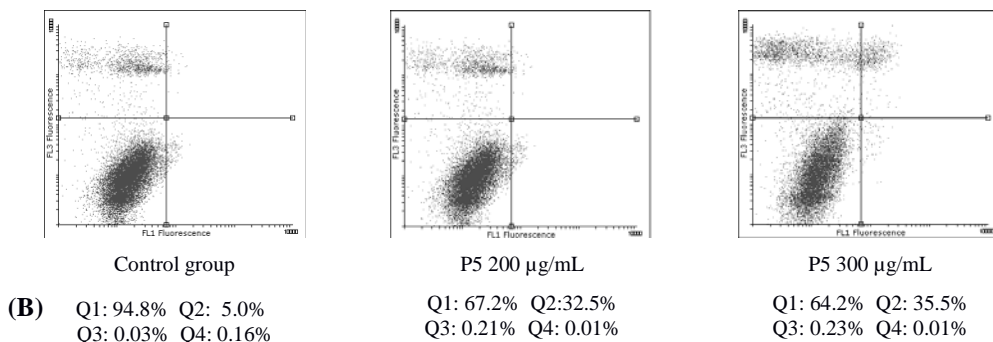


Figure 2. Cytograms of bivariate analysis (Ann V-FITC vs PI) for cell apoptosis and viability identification in Vero cultures treated with different doses of P4 (A) and P5 (B) compounds. Q1 (left-down quarter) denotes viable cells, Q2 (left-up quarter) – dead cells, Q3 (right-up quarter) – apoptotic cells and Q4 (right-down quarter) – preapoptotic cells.

It can be seen, from the above figure (figure 2, A and B), that in the case of normal Vero cells both bioproducts have acted as cytotoxic agents, determining a significant decrease of the cell viability, illustrated by the increase of the dead cell number and the decrease of the alive cells number, the P4 extract inducing a great cytotoxic impact on normal cells, as compared to P5. The apoptosis process was very little amplified in this type of normal cells as compared with the control cultures.

The way of cell malignant transformation from normal to cancerous cell implies a cell to go through complete cycle of progressive changes at cellular, genetic, and epigenetic level that ultimately reprogram a cell to undergo uncontrolled cell division. The complex process of carcinogenesis will assure a number of characteristics (sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis) essential for acquirement of the malignant phenotype (Hanahan & Weinberg, 2011). Due to gained functions, the cancerous cells are able to survive even in the worst conditions or in the case of treatment assault.

Achievement of a targeted therapy, in the conditions of that each individual cancer cell carries no recognizable molecules or structures that make them consistently distinguishable from normal cells (Sonnenschein & Soto, 2011), is a desirable aim.

Addressing to improvement of the antitumoral chemotherapy selectivity, the polyphenols are very promising regarding to prevention of the DNA damages (by their high antioxidant potential) and to impairment of the steady state of the malignant transformed cells.

Vitis vinifera seeds are very rich in bioactive compounds, especially polyphenols, they representing a generous and available source for obtaining new actively biopreparations with agricultural, biomedical and ecological capitalization. In our paper, have been included the results focused on the reactivity of the cell ROS status and apoptosis to the action of some new total polyphenolic bioproducts, extracted from grape seeds.

Analysis of the normal and cancerous cells treated with these compounds doesn't revealed any modifications in the reactive oxygen species levels as compared with the control group. Also, between the cell types weren't registered differences.

The study of cellular apoptosis phenomenon in the same cell cultures has shown that neoplastic cells were more sensitive to the tested compounds as compared with the normal cells. The apoptosis was highly expressed in the case of P4 compound in a dose relationship manner, with the maximum impact at the dose of 300 µg/mL.

Small variations in the oxidative status of the treated cells, as compared with the control group, and apoptosis triggering by polyphenolic compounds proves that cytotoxic effect of the tested compounds is especially due to the proapoptotic effect.

Our investigations, presented in this paper, have revealed that the impact of the polyphenolic extracts, isolated from *Vitis vinifera* seeds, on the normal and cancerous cells is selective, the cytotoxic effect being higher in neoplastic cells than in normal ones.

CONCLUSIONS

Apoptosis triggering is the main effect of the polyphenolic extracts and was highly expressed in the neoplastic cells treated with P4 biopreparation.

Tested biopreparations were cytotoxically selective with a high degree on HeLa cancerous cells.

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LEVELS OF CA125 MARKER IN PATIENTS WITH DIFFERENT HISTOLOGIC TYPES OF OVARIAN CANCER

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Keywords: ovarian cancer, histologic types, CA 125, age at diagnosis

Abstract: Ovarian cancer is one of the most lethal cancers among women worldwide. The most used tumor marker in ovarian cancer is CA 125, elevated levels of CA 125 having been recorded in 80% of patients with advanced stages of the disease. The objective of the present study was to evaluate and compare CA 125 levels in relation with different histologic types of ovarian cancer. We analyzed 124 women diagnosed with ovarian cancer between 2010 and 2016. Four parameters were investigated: age at diagnosis, level of CA 125 at diagnosis, histologic type and location of the tumor. The results show a mean age at diagnosis of 50.29 ± 9.83 years. The serous ovarian cancer was the most frequent histologic type (45.97%). Highest mean levels of CA 125 were recorded in endometrioid (335 U/ml) and serous (792,48 U/ml) tumors. Lower levels were registered in clear cell (139,9 U/ml) and mucinous (169,49 U/ml) tumors.

INTRODUCTION

In developed countries, ovarian cancer is the 5th most common cancer among women and the 7th worldwide (Torre et al., 2015 ; Bray et al., 2015). Around the world almost 239.000 new cases of ovarian cancer are diagnosed each year. With over 151.000 deaths per year worldwide, ovarian cancer is the 8th most fatal female cancer in the world (Torre et al., 2015). In the United States, ovarian cancer is the one of the most lethal malignancy, with over 14.000 deaths in 2015 (Siegel et al., 2015). The new ovarian cancer estimated cases for 2016 in the United States are over 22.000 (Siegel et al., 2016). According to Canadian Cancer Society, in 2015 nearly 2.800 women were diagnosed with ovarian cancer and the number of deaths due to this disease were 1.750 (<http://www.cancer.ca/en/cancer-information/cancer-type/ovarian/statistics/?Region=on>). In Europe, ovarian cancer is the 14th most common malignancy with 65.500 new cases diagnosed in 2012, the highest incidence being in Central and Eastern Europe, with over 28.000 cases (Ferlay et al., 2013). All this data show that, despite the advanced chemotherapy and new surgical procedures, the numbers are still high as well for incidence as for mortality.

The 5-year survival in patients with ovarian cancer is still below 45% (Urban et al., 2016). The majority of ovarian cancers remain clinically silent and almost 75% of patients are diagnosed in advanced stages (Zhu Lan et al., 2016). The cure rate for patients diagnosed in first stage of malignancy is almost 90% (Digant Gupta and Christopher Lis, 2009). Hence, the detection of the disease in the first stage has a decisive impact on survival rate (Negură L. and Negură A., 2016). A variety of biomarkers have been developed for an early detection of the ovarian cancer: CA 19-9, CA 15-3, CA 549, CA 125. From all of these biomarkers, CA 125 is the most utilized and it is regarded as gold standard tumor marker in ovarian cancer (Digant Gupta and Christopher Lis, 2009; Anastasi et al., 2013). CA 125 is a high molecular weight glycoprotein and it is expressed by fetal amniotic and coelomic epithelium (Negură A., 2008). In adult tissues, it is originate from the coelomic and Mullerian epithelia (Digant Gupta and Christopher Lis, 2009). His structure possess 2 major antigenic domains, A and B, which bind monoclonal antibodies OC125 and respectively M11 (Digant Gupta and Christopher Lis, 2009).

Before 2008, the only Food and Drug Administration (FDA) – approved ovarian cancer biomarker was CA 125 (Li et al., 2012). Elevated levels of CA 125 were recorded in 80% of patients with advanced ovarian cancer stages (Li et al., 2012). Also, high levels of cancer antigen 125 were found in benign gynecological diseases and non-gynecological cancers (Zhu Lan et al., 2016). Today, a value of CA 125 below 35 U/mL is considered normal (Digant Gupta and Christopher Lis, 2009). The important role of cancer antigen 125 in early exposure of ovarian cancer it is summarized in reports indicating that elevated levels of CA 125 are registered with 3 months before clinical detection (Digant Gupta and Christopher Lis, 2009 ; Zhang et al., 2015). Several studies indicates the usefulness of this biomarker in monitoring the treatment, disease progression and patient prognosis (Woo Dae Kang et al., 2010).

The NICE guidance indicate that serum CA 125 should be the first test performed on women with suggestive symptoms of ovarian cancer (Moss et al., 2013).

The objective of this study is to evaluate and compare CA 125 levels in relation with different histologic types of ovarian cancer.

MATERIALS AND METHODS

This is a retrospective study of 126 women diagnosed with ovarian cancer between 2010 and 2016. All data were collected from database of the Oncogenetics Department, University of Medicine and Pharmacy Gr.T.Popa Iaşi, and from medical records of the Oncology Clinic, Regional Institute of Oncology, Iaşi. The study had the approval of the Ethical Committee of the University of Medicine and Pharmacy Gr.T.Popa Iaşi. We analyzed four parameters: age at diagnosis, level of CA 125 at diagnosis, histologic type and location of the tumor. All the patients with Krukenberg tumors, benign tumors or metastasis to the ovaries were excluded from the study. The value of CA 125 accepted as normal in this study was 0-35 U/ml. The statistical data are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Of the initial 126 patients, only 124 fulfilled the requirements and were included in the study. The mean and the median age at diagnosis are shown in Table 1.

Table 1. Age at diagnosis for ovarian cancer cases (N=124).

Mean age of diagnosis	50,2984
Median age of diagnosis	51,0000
Std. Deviation	9,83686
Minimum	17,00
Maximum	76,00

According to American Society of Clinical Oncology (ASCO), 68% of women with ovarian cancer are older than 55 and 32% are younger than 55 (<http://www.cancer.net/cancer-types/ovarian-cancer/risk-factors-and-prevention>). In the United States, the median age at diagnosis is 63 (<http://seer.cancer.gov/statfacts/html/ovary.html>). Chan et al., published in 2006, a study on 28.165 American women diagnosed with primary epithelial ovarian cancer. The median age in his report was 64 years (Chan et al., 2006). Recent data published by Praestegaard et al. on 10.601 women from different world regions (Australia, Europe and United States), diagnosed with epithelial ovarian cancer, showed a median age at diagnosis of 57 years (Praestegaard et al., 2016). A case-control research of ovarian cancer in African American women conducted by Schildkraut et al. showed a mean age at diagnosis of $57,4 \pm 11,2$ years (Schildkraut et al., 2014). In Sweden the mean age at diagnosis is $62,4 \pm 7,4$ years (Riman et al., 2004).

In Romania, there are very few available ovarian cancer data. Similar to the present study, a mean age of $51,46 \pm 14,28$ years was found on 82 Romanian women diagnosed with ovarian cancer by Furau et al. (Furau et al., 2011). Another study, published in 2012 by Voicu et al. on 50 Romanian women diagnosed with ovarian cancer, shows a mean age of $54,44 \pm 13,84$ years (Voicu et al., 2012). All these results indicate that we obtained a lower mean age at diagnosis for ovarian cancer than other reports from countries like USA, Australia or Western Europe. On the contrary,

the similarity between our data and those presented in other Romanian studies, may indicate that Romania has a lower mean age at diagnosis for ovarian cancer than Western countries.

From Fig. 1, we can notice that in our study the highest ovarian cancer rate (42,74%) was in women aged 51-60 years. Same results have been obtained by Furau et al. In their study, the highest incidence of ovarian cancer was in 51-60 years group (Furau et al., 2011). According to International Agency for Research on Cancer (IARC), in Romania the peak rate of the ovarian cancer is in 60-69 years group (<http://eco.iarc.fr/eureg/>). In the United Kingdom, 53% of cases diagnosed with ovarian cancer are women aged 65 or more. The highest number of cases (965 cases) are in 65-69 years group (<http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancertype/ovarian-cancer/incidence#heading-One>; Jacobs et al., 2016). The highest percent (23,8%) of new ovarian cancer cases, in the United States, is in 55-64 age group (<http://www.ovariancancer.org/about/statistics/>). In Canada, in 2010, the highest incidence of ovarian cancer, was in women aged 85 and older, with a rate of 46,1 new cases per 100.000 women (Tanya Navaneelan, 2015). Fox et al., developed a study on 112 Canadian women with known diagnosis of high grade serous ovarian cancer. The mean age at diagnosis was $58,04 \pm 10,54$ years and the highest numbers of cases (37,5%) were in 50-59 years group (Fox et al., 2015). Data published in 2010 by Park et al. shows that in 2007 the highest ovarian cancer rate, was in Korean women aged 65-79 years (Park et al., 2010). In Australia, in 2016 it is expected the incidence of ovarian cancer will increase with age, until age group 65-69 (<https://ovarian-cancer.canceraustralia.gov.au/statistics>). Our results in mean and group age show a lower age at diagnosis than other Western countries, but very similar with studies developed in Romania. At the same time, our records are inconsistent with data published by IARC. Further studies are needed to confirm this facts.

Table 2. Location of the tumor for ovarian cancer cases (N=124).

Location of the tumor					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Unspecified	36	29,0	29,0	29,0
	Left	22	17,7	17,7	46,8
	Right	29	23,4	23,4	70,2
	Bilateral	37	29,8	29,8	100,0
	Total	124	100,0	100,0	

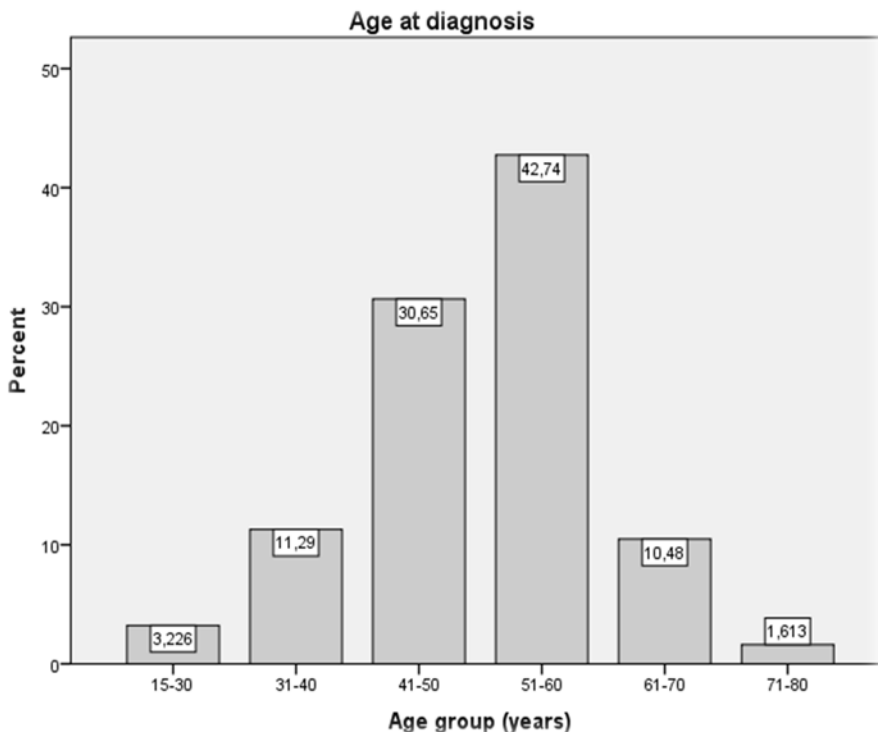


Figure 1. Structure of our patients lot by groups of age.

From table 2 we can observe that, the right ovary has developed cancer in 29 cases (23,4%). In 36 cases (29%), we were unable to establish the correct location of the tumor. Bilateral location reached the maximal frequency (29,8%). The lowest occurrence was in unilateral left ovarian cancer (17,7%).

Fig. 2 indicates the main histologic types found in our study. The serous ovarian cancer was the most frequent histologic type (45,97%). Unfortunately, more than 18% of all cases had an unspecified histologic type.

Data published by Oberaigner et al., from 69 European cancer registries, reveal that overall proportions of the serous, mucinous, germ cell, other tumors and not otherwise specified, were 45,8%, 10,1%, 1,5%, 35,9% and 6,8% respectively. The highest rate of serous ovarian cancer were in Iceland (60.4%). The largest number of mucinous ovarian cancers were in Italy with 17,9% (Oberaigner et al., 2012). Similar data were obtained in Sweden by Riman et al., on 655 cases of epithelial ovarian cancer. They have identified 337 cases of serous ovarian cancer (51%), 60 cases of mucinous tumors (9%), 180 cases of endometrioid tumors (27%), 43 cases of clear-cell tumors (7%) and 35 cases of undifferentiated or others tumors (5%). The mean age at diagnosis for every histologic type was: $62,6 \pm 7,3$ years for serous tumors, $62,5 \pm 7,8$ years for mucinous tumors, $61,6 \pm 7.6$ years for endometrioid tumors and $61,2 \pm 7,2$ years for clear-cell tumors (Riman et al., 2004).

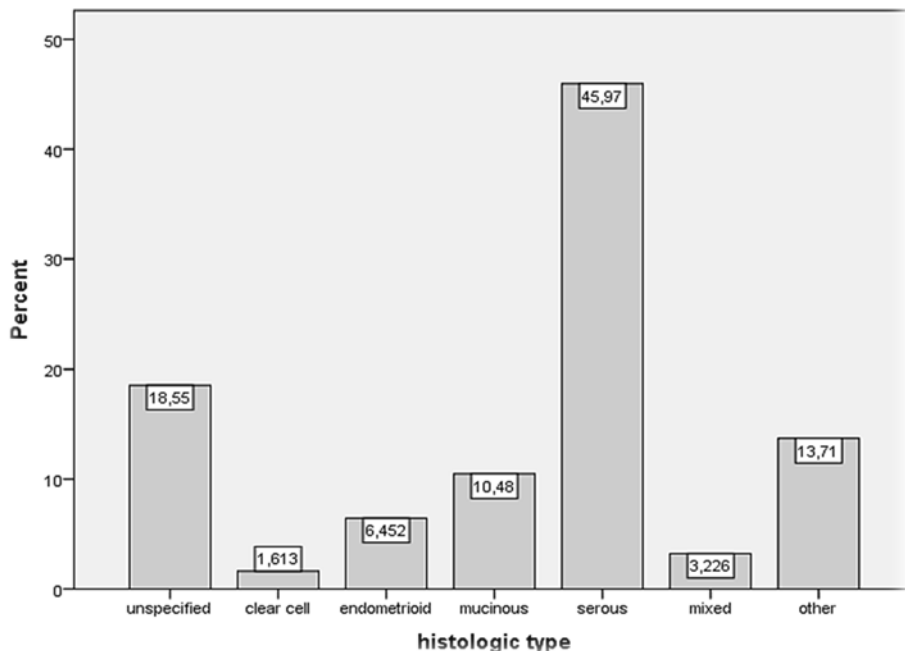


Figure 2. Structure of our patients lot by histologic subtypes of ovarian cancer.

In our study, the mean age for serous tumors was $48,91 \pm 8,51$ years. It is clear that our data indicate a similar proportion of histologic subtypes of ovarian cancer with other studies, but the mean age of serous tumors, in our study, is lower.

Fig. 3 shows the mean levels of CA 125 in different histologic types of ovarian cancer. Much higher values were obtained by Thakur et al., in a study on 40 patients with epithelial ovarian cancer. They presented high mean levels of CA 125 in serous adenocarcinoma ($1571 \pm 121,5$ U/ml) and endometrioid carcinoma (2853 ± 136 U/ml). Mucinous adenocarcinoma and clear cell carcinoma had lower mean levels of CA 125: 775 ± 78 U/ml and 60 U/ml (Thakur et al., 2003). Our levels of CA 125 are much lower than those presented by Thakur et al. and the proportion is similar, except for endometrioid carcinoma. They presented very high mean levels of CA 125 in endometrioid carcinoma, but, in our study the highest mean level of CA 125 was in serous adenocarcinomas. Lower levels of CA 125 were recorded in mucinous and clear cell carcinoma, but all these levels were much higher than 35 U/ml. Similar results to ours, were obtained by Będkowska et al. in a reasearch on 110 epithelial ovarian cancer patients. In their case, the highest levels of CA 125 were in serous epithelial group (the median of CA 125 levels was 171,24 U/ml). The median of CA 125 levels in endometrioid epithelial group, was 114,24 U/ml (Będkowska et al., 2015).

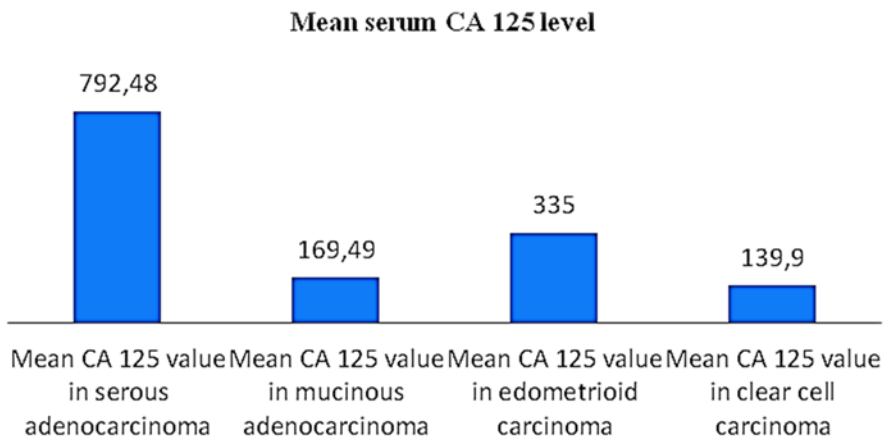


Figure 3. Mean levels of CA 125, by histological types of ovarian carcinoma.

This evidence confirm the utility of CA 125 in early diagnosis of ovarian cancer. Detection of high levels of CA 125 in women with no clinical signs, should be followed by other investigation, in order to exclude an ovarian malignancy. Also, our study suggests that CA 125 may have a role in early prediction of histological sub-types of ovarian cancer but additional studies should be performed to evaluate this possibility.

CONCLUSIONS

Ovarian cancer remains a challenge for the medical world. Our data have a proportional similarity with other studies, except for the age at diagnosis. In our study, the mean and median age at diagnosis (50,29/51 ± 9,83 years) seems to be lower than other reports. Countries like USA, Canada or Western Europe have a mean age at diagnosis over 60 years. Same differences are in age groups: our data indicates the highest ovarian cancer incidence in 51-60 years group.

The mean serum CA 125 levels are very high, especially in ovarian serous adenocarcinoma. The mucinous and clear cell tumors have a lower level of serum CA 125 than serous or endometrioid adenocarcinoma, which is consistent with literature data. Although numerous new biomarkers have been identified, CA 125 remains the gold standard biomarker in early detection of ovarian cancer.

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EVALUATION OF LIVER BIOMARKERS AFTER LAPAROSCOPIC CHOLECYSTECTOMY IN PATIENTS UNDER 60 YEARS

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Keywords: laparoscopic cholecystectomy, pneumoperitoneum, aspartate transaminase (AST), alanin transaminase (ALT), bilirubin.

Abstract: Laparoscopic cholecystectomy (LC) is gold standard treatment for cholelithiasis. This surgical procedure requires insufflation of carbon dioxide into peritoneal cavity. Intra-abdominal pressure increases, causing disturbances on blood flow of the portal vein with secondary liver injury. In this study we evaluated the hepatic function in 147 patients who underwent laparoscopic cholecystectomy. Three liver biomarkers have been measured 24 hours before and 24 hours after the surgery: Aspartate transaminase (AST), Alanine transaminase (ALT) and total bilirubin. The results show significantly increased levels of all 3 biomarkers, 24 hours after the surgery. We also found a correlation between surgery duration and levels of serum AST, ALT and bilirubin. The highest levels of hepatic enzymes and total bilirubin were recorded in patients with maxim of minutes (80-90) under pneumoperitoneum.

INTRODUCTION

Cholelithiasis is one of the most common diseases of digestive tract. In USA it affects more than 20% of the population above 40 years. Early diagnosis and immediate treatment can prevent severe complications such cholecystitis, acute pancreatitis or peritonitis. In the last decades, the gold standard procedure for surgical treatment of cholelithiasis was represented by laparoscopic cholecystectomy (LC) (Torres et al., 2009 ; Duca S., 2001). Nowadays, almost 90% of all cholecystectomies are performed laparoscopically (Eryilmaz et al., 2012). LC has numerous advantages over classic cholecystectomy like shorter convalescence, rapid recovery, less postoperative pain and less visible scars (Lai H. et al., 2014 ; Agresta et al., 2014).

Despite all these advantages, numerous complications were reported, some of them related to carbon dioxide pneumoperitoneum (CDP). There are reports about pneumoperitoneum effects on cardiovascular and respiratory system. Several studies have demonstrated the association between carbon dioxide pneumoperitoneum and hepatic injury. Elevated levels of aspartate transaminase (AST) and alanine transaminase (ALT) were noticed in patients who underwent LC (Baris Eryilmaz et. all, 2012 ; Sharma et al., 2015 ; Krishnegowda et al., 2016). Experimental models have shown that intra-abdominal pressure of 12-14 mmHg of CO₂ can reduce portal blood flow and cause hepatic ischemia. The mechanism of liver ischemia is related by the inflation and deflation of pneumoperitoneum. This ischemia-reperfusion phenomenon cause hepatic injury with release of transaminases. Leukocytes and platelets are permanently adherent to endothelium of hepatic sinusoids which indicate microcirculation abnormalities (Bostanci et al., 2010; Sandhu et al., 2009; Ypsilantis et al., 2016).

The aim of this study is to evaluate the impact of CDP on hepatic injury by comparing preoperative and postoperative levels of serum biomarkers in in patients under 60 years old who underwent LC.

PATIENTS AND METHODS

The study was realized in Surgery Department of C. F. Hospital, Iasi. Between 2014 and 2016 a total 306 LC were performed. The inclusion criteria in study were: age(18-59 years), gallbladder disease and normal values of liver biomarkers 24 hours before the surgery. Patients with cardiac or hepatic dysfunctions, high levels of preoperative liver enzymes, common bile duct stones, positive serology for hepatitis B or C virus, conversion to open cholecystectomy were excluded. For each patient, the diagnosis was confirmed by a physical examination and abdominal ultrasound. The LC was performed under general anesthesia. All the operations were performed using the standard American technique. The carbon dioxide pneumoperitoneum was installed with Veress needle. During the surgery, 12-14 mmHg pressure of pneumoperitoneum was maintained with an automatic insufflator. The following liver biomarkers were analyzed: total bilirubin, aspartate transaminase (AST) and alanine transaminase (ALT). Blood samples were taken from each patient by peripheral vein with 24 hours before surgery. Another blood sample was taken 24 hours after the surgery. In both cases, all samples were analyzed in hospital laboratory and the levels of AST, ALT and serum bilirubin were recorded. The normal values of hepatic biomarkers used in this study are: for AST, 5-40 U/L; for ALT, 5-35 U/L; for total bilirubin, 0.2-1.1 mg/dL. The duration of surgery was recorded from the installation of pneumoperitoneum to the time of desufflation and trocars removal. During the postoperative period no other medication was prescribed, but the intravenous antibiotics and

analgesics. All data were expressed as mean ± standard deviation (SD). We also used paired *t*-test for levels of liver biomarkers before and after the surgery. A *P* value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

From a total of 306 LC performed, 199 (65,03%) were patients under 60 years old. After we applied the inclusion and exclusion criteria, only 147 patients were included in the study. The age, sex and time of surgery duration are shown in the Table 1 and Table 2.

Table 1. The distribution of sexes

Table 1					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Female	124	84,4	84,4	84,4
	Male	23	15,6	15,6	100,0
	Total	147	100,0	100,0	

The gender was dominated by female with 84,4% and the mean age of the patients was $46,85 \pm 8,85$ years. The mean duration of the surgery was $57,57 \pm 13,12$ minutes. We have chosen patients under 60 years old for a better perspective of the pneumoperitoneum influence on normal hepatic function. The proportion of patients over 60 with comorbidities was much higher than those under 60 years old. This polymorbidity could interfere with the results.

Table 2. Age and surgery duration in minutes

Table 2					
	N	Minimum	Maximum	Mean	Std. Deviation
Age	147	19,00	59,00	46,8503	8,85140
Surgery duration	147	40,00	90,00	57,5714	13,12323
Valid N (listwise)	147				

From fig. 1 we can observe that the mean preoperative AST levels (normal value:5-40 U/L) were $28,01 \pm 3,69$ U/L and after the surgery, values have raised to $71,1 \pm 12,06$ U/L. Similar data are shown for ALT ($26 \pm 2,67$ U/L before and $70,1 \pm 11,85$ U/L after the surgery) and serum total bilirubin ($0,57 \pm 0,1$ mg/dL preoperative and $1,74 \pm 0,12$ mg/dL postoperative). Sefik Hasukic, in a prospective study, evaluated the effects of pneumoperitoneum on hepatic function. His data showed that in the first 48 hours postoperatively, levels of AST and ALT were

significantly increased (Hasukic et al., 2005). Rikki Singal et al. found high levels of AST, ALT and bilirubin 24 hours after LC. In their study, the mean postoperative values for AST were $72,9 \pm 13,1$ and $72,4 \pm 12,9$ for ALT. They found a significant difference ($P=0,0001$) between preoperative and postoperative values of hepatic transaminases (Singal et al., 2015).

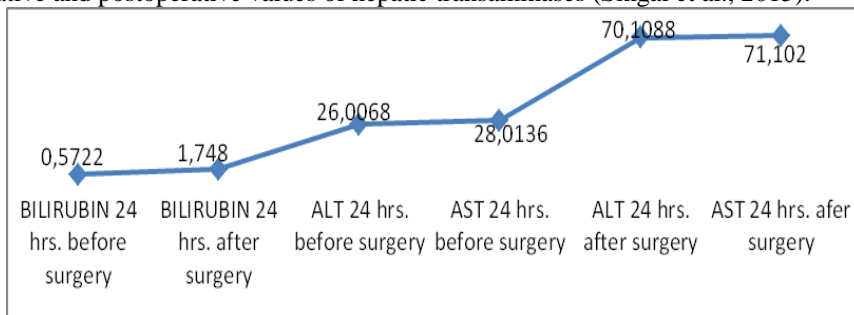


Fig. 1. The mean levels of the liver biomarkers 24 hrs. before surgery and 24 hrs. after surgery

The paired *t*-test comparison between preoperative and postoperative levels of AST, ALT and serum bilirubin shows that the data are statistically significant ($P<0,01$). The mean difference between postoperative and preoperative level of AST is $43,08 \pm 12,6$ U/L. For ALT and serum bilirubin, the mean difference is $44,1 \pm 12,09$ U/L respectively $1,17 \pm 0,17$ mg/dL. The confidence interval is similar for AST and ALT (41,03-45,14 and 42,13-46,07)(Table 3).

Table 3. Comparison between preoperative and postoperative levels of AST,ALT,bilirubin

		Paired Differences				t	df	P value	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1	Postoperative AST	43,088	12,61	1,0401	41,03	45,144	41,4	14	0,0
	Preoperative AST	44	111	5	275	12	25	6	00
Pair 2	Postoperative ALT	44,102	12,09	0,9976	42,13	46,073	44,2	14	0,0
	Preoperative ALT	04	593	6	033	75	06	6	00
Pair 3	Postoperative BILIRUBIN	1,1762	,1761	0,0145	1,147	1,2049	80,9	14	0,0
	Preoperative BILIRUBIN	6	2	3	55	7	77	6	00

From Fig. 2 and Fig. 3 we can observe the correlation between duration of surgery (in minutes) and levels of serum AST, ALT and bilirubin. Patients with minimum of minutes (40 min.) under the pneumoperitoneum have lower values of hepatic enzymes and bilirubin. The highest levels of hepatic biomarkers were recorded at the patients with surgery duration between 80-90 minutes.

In our study, the mean levels of AST, ALT and bilirubin for patients with 40 minutes under pneumoperitoneum were: for AST $57 \pm 1,73$ U/L, for ALT $55,66 \pm 1,52$ U/L and for bilirubin $1,57 \pm 0,07$ mg/dL. On the contrary, the mean levels of the patients with 90 minutes under

pneumoperitoneum are much higher: for AST $111,66 \pm 3,78$ U/L, for ALT $109,33 \pm 4,04$ U/L and for bilirubin $2,03 \pm 06$ mg/dL.

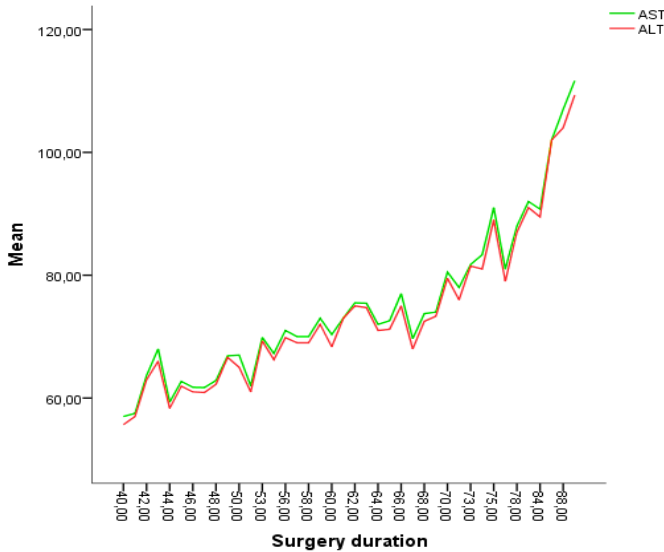


Fig. 2. The correlation between surgery duration and postoperative AST, ALT levels

The duration of surgery, in our study, has a clear correlation with levels of hepatic biomarkers. Hao Lai has concluded in a meta-analysis on 11 comparative studies, that the duration of CO₂ pneumoperitoneum may be associated with hepatic injury (Lai et al., 2014).

From this study, it is clear that liver biomarkers are elevated after the laparoscopic surgery. Our data show that the level of pneumoperitoneum pressure at 12-14 mmHg may have a significant impact in levels of hepatic enzymes and bilirubin. A study conducted by Makoto Yoshida on a porcine pneumoperitoneum model, showed that the portal vein pressure increased to 15 mmHg for the pneumoperitoneum group, but no significant difference in the levels of serum aminotransferase were recorded (Yoshida et al., 2010). Erdal Birol Bostanci has conducted in 2009 an experimental study in a rat model. He applied carbon dioxide pneumoperitoneum to a number of rats. The results showed that the insufflation of CO₂ in the peritoneal cavity to create pneumoperitoneum deteriorates hepatic cell integrity, with an increase in liver enzymes and alteration of the hepatic microcirculation. Levels of AST recorded in their study were $347,4 \pm 48$ and $67,6 \pm 8$ for ALT (Bostanci et al., 2009). Due to this postoperative complication of LC, surgeons have developed new methods with low pressure pneumoperitoneum. In this way, Barczynski and Herman compared low pressure (7mmHg) and standard pressure (12mmHg) pneumoperitoneum in laparoscopic cholecystectomy. They concluded that a low pressure peritoneum is superior to standard pressure in terms of the quality of life within 5 days after the operation (Barczynski, 2003).

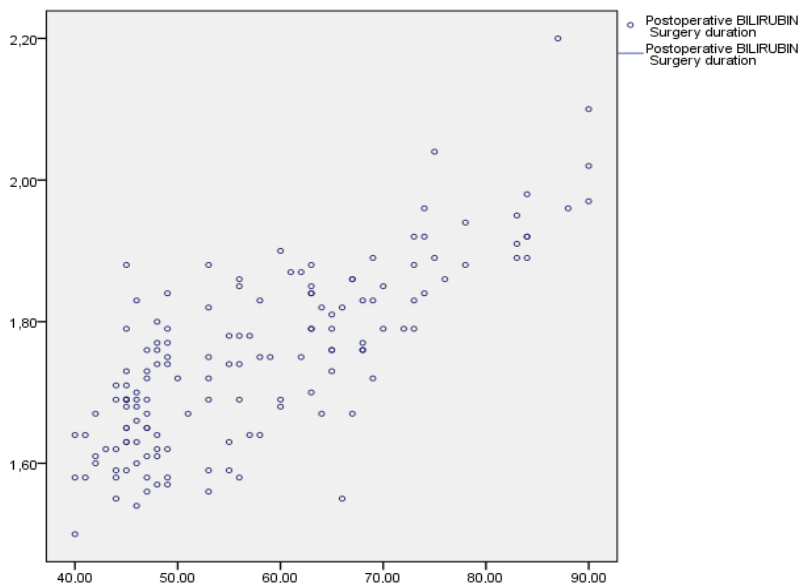


Fig. 3. The correlation between surgery duration and postoperative bilirubin levels

There are also described methods without pneumoperitoneum. Daijo Hashimoto has developed a model of LC by traction on the abdominal wall with a hanger lifting method. (Hashimoto et. al., 1993).

CONCLUSIONS

The pneumoperitoneum seems to have an important role in increasing postoperative levels of serum AST, ALT and bilirubin. The 12-14 mmHg standard pneumoperitoneum pressure is not the ideal pressure to perform LC. The duration of surgery alongside high levels of pneumoperitoneum pressure may affects liver functions. Alternatives methods of laparoscopic surgery, with low pressure peritoneum should be developed.

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BLOOD PROFILE OF WEST AFRICAN DWARF GOATS FED PROVITAMIN A CASSAVA PEEL-CENTROSEMA LEAF MEAL BASED DIETS

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Abstract: The study was conducted to investigate the effects of feeding varying levels of provitamin A cassava peel-centrosema leaf meal at 0%, 10%, 20% and 30%, respectively as supplement to *Panicum maximum*, on the haematological and serum biochemical parameters of West African Dwarf (WAD) goats. The study lasted for 97 days during which haematological and serum biochemical parameters were evaluated in 36 bucks, which were randomly divided into four (4) groups of 9 goats each, with three goats constituting a replicate in a completely randomized design pattern. Blood samples were drawn from each animal on the last day of the trial and evaluated for haematological and biochemical profile analyzed statistically. The packed cell volume (PCV), haemoglobin (Hb), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) significantly ($P < 0.05$) ranged from 28.87% to 32.91%, 10.77 to 13.23 g/dl, 31.05 to 34.25% and 19.04 to 19.04 fl respectively, while red blood cell (RBC) and mean corpuscular haemoglobin (MCH) were significantly ($P > 0.01$) similar and ranged from 10.97 to 11.01 $\times 10^9$ /ul and 6.13 to 6.34 pg respectively. White blood cells and the differentials increased significantly ($P < 0.05$) with increasing levels of the test ingredients. Total protein, albumin and globulin significantly ($P < 0.05$) ranged from 66.21 to 73.32 g/l, 32.20 to 34.33 g/l and 34.01 to 38.99 g/l respectively. Serum creatinine increased significantly ($P < 0.05$) as the level of test ingredient increased while serum urea and AST decreased significantly ($P < 0.05$) as the levels of the test ingredient increased from A to D. Cholesterol however showed significant difference ($P < 0.05$) but did not follow any specific trend with increasing or decreasing levels of provitamin A cassava peel-centrosema leaf meal. The study revealed that provitamin A cassava peel-centrosema leaf meal in the diets of West African Dwarf goats had no deleterious effects on the haematological and serum biochemical parameters of WAD goats and could therefore be included in ruminant diets up to 30%.

INTRODUCTION

The provitamin A cassava have been developed either through traditional plant-breeding or bioengineering (Sayre *et al.*, 2011). The new provitamin A cassava varieties have potential of providing up to 25% of daily Vitamin A requirements of children and women (Aniedu and Omodamiro, 2012). Cassava with higher levels of provitamin A can help reduce vitamin A deficiency among undernourished communities that rely upon cassava for sustenance. Provitamin A cassava varieties are very high in carotenoids. More recently, protective effects of carotenoids against serious disorders such as cancer (Donaldson, 2004), heart disease (Sesso, 2003) and degenerative eye disease (Mozaffarieh *et al.*, 2003) have been recognized, and have stimulated intensive research into the role of carotenoids as antioxidants and as regulators of the immune response system. These numerous advantages has led to high demand for the provitamin A cassava for different human or industrial uses thereby enhancing the availability of the peels which are grossly underutilized and were hitherto discarded as waste. The provitamin A cassava peels are made up of mainly polysaccharides and carotenoids; hence holds inestimable potentials as energy and vitamin A sources for goats. However, due to its crude protein deficiency, the need to supplement with readily available noncompetitive unconventional protein source like *Centrosema pubescens* leaf meal becomes imperative. Nworgu and Egbunike (2013) reported 23.24% Crude protein in *Centrosema pubescens* leaf meal; hence being a potential protein source in goat production.

Evaluation of the blood profile of animals may give some insight as to the potentials of a dietary treatment to meet the metabolic needs of the animal. Church *et al.* (1984) noted that dietary components have measurable effects on blood constituents such that significant changes in their values can be used to draw inference on the nutritive value of feeds offered to the animals. Haematological and serum biochemical studies are widely used for the diagnosis of animal diseases as well as investigation of blood damage and feed toxicity and protein quality (Jiwuba *et al.*, 2016). Blood profile studies are important since blood is the major transport system of the body and evaluations of the blood profile usually furnish vital information on the body's response to injury of all forms, feed quality and toxicity, the authors concluded. However, the assertion of Ikhimoya and Imasuen (2007) that most of the available information on haematological parameters of goats in

the humid tropics is based on disease prognosis from the region. Thus, data on haematological and serum biochemical profile of West African dwarf (WAD) goat offered agricultural biotechnological byproduct and leaf meal from non-conventional sources are scanty.

The sourcing for readily, locally available and nutritionally viable feed ingredients to enhance food production stimulated this research which aimed at evaluating the efficacy of provitamin A root cassava peel – centrosema leaf meal based diets on growth and haematological and serum biochemical parameters of West African dwarf goats.

MATERIALS AND METHODS

The experiment was carried out at the sheep and goat Unit, Federal College of Agriculture, Ishiagu, Ivo L.G.A., Ebonyi state, Nigeria. The College is located at about three kilometers (3km) away from Ishiagu main town. The College is situated at latitude 5.56°N and longitude 7.31°E, with an average rainfall of 1653 mm and a prevailing temperature condition of 28.50°C and relative humidity of about 80%. Fresh provitamin A cassava peels varieties (TMS011368, TMS011412 and TMS1371) were obtained from National Root Crops Research Institute, Umudike, Abia State, Nigeria. The peels were subsequently dried to about 10% moisture content before milling and used in the formulation of provitamin A cassava peel - centrosema leaf meal based diets. Fresh green *Centrosema pubescens* leaves were harvested within the College. The *Centrosema pubescens* were shade-dried in batches, milled and also used at different levels in the formulation of provitamin A cassava peel - centrosema leaf meal based diets.

Thirty six (36) WAD goats of about 8–10 months of age and averaging 7.19kg in weight were selected from the College herd for this experiment. The goats were randomly divided into four (4) groups of nine (9) animals each with 3 goats constituting a replicate. The groups were randomly assigned the 4 experimental diets (A, B, C and D) in a completely randomized design (CRD). The animals were housed individually in well ventilated cement floored pens equipped with feeders and drinkers. Each animal received a designated treatment diet in the morning for 97 days. Feed offered was based on 3.5% body weight per day; the animals in addition were fed 2kg wilted *Panicum maximum* later in the day. Regular access to fresh drinking water was made available.

Experimental diets designated as A, B, C and D were formulated from provitamin A cassava peel, brewers dried grain, palm kernel meal, wheat offal, *Centrosema pubescens* leaf meal, bone meal, molasses and salt. Diet A served as a positive control and contained 0% of *Centrosema pubescens* leaf meal. Diets B, C and D contain 10%, 20% and 30% inclusion levels *Centrosema pubescens* leaf meal respectively as illustrated in Table 1. All feeds and test ingredients were analyzed for proximate compositions using the method of AOAC (2000). Gross energy was determined according to Nehring and Haelein (1973).

Table 1: Percentage Composition of the provitamin A cassava peel-centrosema leaf meal based diets

Ingredients (%)	Dietary levels (%)			
	A	B	C	D
Provitamin A Cassava peel	40.00	40.00	40.00	40.00
<i>Centrosema pubescens</i> leaf meal	0.00	10.00	20.00	30.00
Brewers dried grain	38.00	28.00	18.00	8.00
Palm kernel cake	18.00	18.00	18.00	18.00
Bone meal	2.00	2.00	2.00	2.00
Molasses	1.50	1.50	1.50	1.50
Common salt	0.50	0.50	0.50	0.50
Total	100	100	100	100

Five ml of blood samples were drawn from each animal on the last day of the study. The goats were bled through the jugular vein. The samples were separated into two lots and used for haematological and biochemical determinations. An initial 2.5 ml was collected from each sample in labelled sterile universal bottle containing 1.0 mg/ml ethyldiamine tetracetic acid (EDTA) and used for haematological analysis. Another 2.5 ml was collected over anti-coagulant free bottle and used for the serum biochemical studies. Serum biochemistry and haematological parameters were measured using Beckman Coulter Ac-T10 Laboratory Haematology Blood Analyzer and Bayer DCA 2000+ HbA1c analyzer, respectively. Mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentrations (MCHC) were calculated.

The results were analyzed using the Special Package for Social Sciences Window 17.0. One-way analysis of variance (ANOVA) was employed to determine the means and standard error. Treatment means were compared using Duncan's new multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The chemical compositions of the experimental diets, provitamin A cassava peel meal (PCPM) and *Centrosema pubescens* leaf meal (CPLM) used in this study is presented in table 2. The proximate

analysis revealed the presence of dry matter, crude protein, crude fibre, ether extract, nitrogen extract and ash. The proximate values for the provitamin A cassava peel meal showed a higher crude protein (CP), ash and ether extract (EE) and lower crude fibre (CF) values compared to the reports of Ahamefule *et al.* (2005) for cassava peel meal. The differences could be attributed to the improvements that have been carried on the yellow root cassava. The proximate compositions of the *centrosema pubescens* leaf meal in this study are comparable with the findings of Nworgu and Egbunike (2013) for the same leaf meal. The dry matter levels of the test diets (B, C and D) compared favourably well with the control diet (A). The CP, ash and ether extract of the test diets were higher than the control diet and tended to increase with the increasing levels of CPLM in the diets. The fibre content on the other hand is higher in the control diet and tended to decrease with increasing levels of CPLM. The nitrogen free extract and gross energy did not show any specific trend among the diets.

Table 2: The chemical compositions of yellow root cassava peel, *Centrosema* leaf meal and provitamin A cassava peel-centrosema leaf meal based diets

Parameters	Dietary levels (%)				PCPM	CPLM
	A	B	C	D		
Dry matter (%)	91.32	91.54	91.37	91.74	90.28	86.03
Crude protein (%)	12.11	12.78	13.41	14.32	9.23	20.44
Crude fibre (%)	14.36	14.65	13.13	13.07	12.93	10.32
Ether extract (%)	4.83	5.11	5.97	5.99	3.41	2.09
Ash (%)	9.01	9.63	10.17	10.19	9.74	6.95
Nitrogen free extract (%)	51.01	49.37	48.69	48.17	55.67	44.22
Gross energy	3.90	3.92	3.91	3.95	3.71	3.64

PCPM = Provitamin A cassava peel meal; CPLM = *centrosema pubescens* leaf meal

The haematology of West African dwarf (WAD) goat fed provitamin A cassava peel-centrosema leaf meal based diets is presented in Table 3. Packed cell volume, haemoglobin, mean cell haemoglobin concentration, mean cell volume, white blood cell neutrophil and lymphocyte differed ($P < 0.005$) while red blood cell and eosinophil were similar ($P < 0.05$) across the treatment groups. The packed cell volume (PCV) values (%) for the treatment groups fell within the normal range for WAD goats (21-35%), as reported by Daramola *et al.* (2005) and differed significantly ($P < 0.05$) among the treatment groups. PCV is generally used as an index of toxicity and its value is influenced by breeds, age and sex. The significant increase in the concentration of PCV in the blood usually would suggest the absence of a toxic factor like haemagglutinin which have adverse effect on blood formation (Jiwuba *et al.*, 2016). *Centrosema pubescens* had been reported to contain 20.01mg/kgDM iron (Nworgu and Egbunike, 2013) and provitamin A cassava implicated to contain high level of vitamin A; a fat-soluble vitamin, involved in a number of physiological processes like hematopoiesis (Guimarães *et al.*, 2014). It is possible that traces of iron and the vitamin A still abiding in the treatment diets may perhaps have been responsible for the improved values.

The haemoglobin (Hb) value of the treatment groups differed ($P < 0.05$) significantly with diet A animals having the lowest (10.77g/dl) and diet D animals the highest value of 13.23g/dl. The Hb concentration compared favourably with the normal range of 7-15g/dl reported by Daramola *et al.* (2005) for WAD goats. The high level of Hb of animals on diet D relative to other treatment groups may imply that the dietary protein was of higher quality, probably due to 30% fortification of the diets with *Centrosema pubescens* leaf meal. This agrees with the observation of Esonu *et al.* (2003)

that one of the possible sources of cheap protein in the diets is the leaf meal of some tropical legumes. Diets containing poor quality protein would usually influence poor transportation of oxygen from the respiratory organs to the peripheral tissues (Robert *et al.*, 2000).

Mean cell haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) of the WAD goats fed provitamin A cassava peel-centrosema leaf meal based diets differed significantly ($P<0.05$) while mean cell haemoglobin (MCH) values were similar ($P>0.05$) among the treatment groups. All the values however fell within the reference range for goats as reported by Fraser and Mays (1986). The normal range of MCV, MCHC and MCH recorded in this study for the WAD goats gave a clear indication of the absence of anaemia among the experimental groups. In humans, a close relationship between vitamin A deficiency and anemia has been long recognized (Semba and Bloem, 2002).

White blood cells (WBC) differed significantly ($P<0.05$) among treatment groups. The values obtained in this study ($8.82-11.69 \times 10^9/l$) were within normal range of $6.8-20.1 \times 10^9/l$ for WAD goats and $4-13 \times 10^9/l$ as reported by Daramola *et al.* (2005) and Fraser and Mays (1986) respectively. WBC of the goats showed an increasing value in relation to an improvement of the immunological system. Vitamin A has been extensively studied for its influence on immunity due to its requirement for normal functioning of the immune system. Vitamin A deficient people are more prone to more severe infections and have a higher mortality than vitamin sufficient people (Beaton *et al.*, 1992).

Lymphocyte and neutrophil differed ($P<0.05$) significantly and however fell within the reported reference range by Fraser and Mays (1986), while eosinophil were similar ($P>0.05$). The circulating lymphocytes in WAD goat, like in other ruminants were higher than Neutrophils. The percentage distribution of the White blood cell differentials in all the animals is in agreement with the reported values of Belewu *et al.* (2006). The linear increase in the WBC differentials could probably show that animals on diets A to D maintained an active immune system that defends the body against infection, allergic reactions, parasites and antigens; thus highlighted the activities of carotenoid in the development of immune system in the WAD goats. This conforms to Lin *et al.* (2006) who stated that Vitamin A is necessary for increasing the immunity through the production of antibodies and its deficiency would reduce immune response and increase susceptibility to infection. In addition, studies have found that beta-carotene supplementation substantially increases the proliferation of lymphocytes, a marker of immune function and immune cell surveillance (Moriguchi *et al.*, 1996).

Table 3: Haematology of West African dwarf (WAD) goat fed provitamin A cassava peel-centrosema leaf meal based diets

Parameters	Dietary levels				SEM	Normal range
	A	B	C	D		
Packed Cell Volume (%)	28.87 ^c	30.35 ^b	30.53 ^b	32.91 ^a	2.49	22 - 38 (%)
Red Blood Cell $\times 10^6/l$	10.94	10.71	10.97	11.01	0.41	8-18($\times 10^6$ / μL)
Haemoglobin g/dl	10.77 ^d	11.63 ^c	12.40 ^b	13.23 ^a	0.48	8 - 12 g/dl
MCHC (%)	31.05 ^b	33.68 ^a	33.79 ^a	34.25 ^a	1.52	30-36 ((g/dl)
Mean cell Haemoglobin (pg)	6.13	6.27	6.29	6.34	0.19	5.2 - 8 (pg)
Mean cell volume (fl)	17.04 ^b	17.11 ^b	18.37 ^a	19.04 ^a	0.97	16 - 25 (fl)
White Blood Cell ($\times 10^9/l$)	8.82 ^d	9.38 ^c	10.50 ^b	11.69 ^a	0.41	4-13($\times 10^3/\mu L$)

Lymphocyte (%)	61.50 ^c	64.13 ^b	64.50 ^b	67.13 ^a	4.53	50 - 70 (%)
Neutrophil (%)	32.78 ^c	34.81 ^b	35.98 ^b	38.14 ^a	2.35	30 - 48 (%)
Eosinophil (%)	1.75	1.50	1.25	1.00	0.09	1 - 8 (%)

^{a, b, c, d} means in the row with different superscripts are significantly different ($P < 0.05$)

MCHC = Mean Cell Haemoglobin Concentration

The blood biochemistry of West African dwarf (WAD) goat fed provitamin A cassava peel-centrosema leaf meal based diets is presented in Table 4. Total protein, albumin, globulin and creatinine were significantly ($p < 0.05$) influenced by the diets and tended to increase with an increasing levels of the test ingredients. Urea and AST also were significantly ($p < 0.05$) affected and followed a specific pattern decreasing with increasing levels of the test ingredients. Bilirubin, ALT and ALP were not affected ($p > 0.05$) by the treatment diets. Cholesterol however differed significantly ($p < 0.05$) but did not follow any specific trend. The total protein, albumin and globulin were significantly ($P < 0.05$) influenced by the treatment diets. The total protein fell within the normal range reported for healthy goats, indicating that the animals did not survive at the expense of body reserves. However, the statistically significant ($P < 0.05$) differences observed among the treatment animals may related to reports of Jiwuba *et al.* (2016) that the serum protein is related to the amount of available dietary protein. The albumin and globulin values are slightly comparable to values reported by Oni *et al.* (2012) for WAD goats fed dried cassava leaves-based concentrate diets and fell within the reference range for healthy goats; an indication of proper functioning of the liver and high immunity response of the experimental animals respectively (Jiwuba *et al.* 2016). The normal range observed in this study for the total protein, albumin and globulin of WAD goats suggested that provitamin A cassava peel and *centrosema pubescens* leaf meal contained a tolerable levels of tannins known to diminish nutrient permeability in gut walls as well as increase excretion of endogenous protein which is subsequently passed out in the faeces and so may not alter protein metabolism (Mitjavila *et al.*, 1977).

The creatinine and serum urea showed a significant ($P < 0.05$) difference among the treatment groups. Creatinine was highest (1.34mg/dl) in diet D animals and lowest (1.10mg/dl) in diet B animals and fell within the normal range (0.7-1.5mg/dl) of apparently healthy goats as reported by Fraser and Mays (1986); suggesting that there was no wasting or catabolism of muscle and that the animals did not survive at the expense of body reserve. Hence, Prvulovic *et al.* (2012) noted that creatinine level is direct correlation with muscle mass and kidney function in animals. This indicated that dietary protein was well used by the goats. The serum urea concentration in this study tended to decrease with increasing levels of the test ingredient with animals on diet A having the highest value and the lowest on diet D and however fell within reference range for apparently healthy goat. Increase in serum urea concentration may suggest an increase in ornithine, carbonyl transferase and arginase, which may indicate kidney damage (Ajabonna *et al.*, 1999). The normal range of values implied therefore that the dietary proteins of the test diets were better utilized.

The diets in this present study did not significantly ($P > 0.05$) influence the bilirubin and cholesterol values in the serum of the WAD goats, hence indicated the safety of the diets as supplements for WAD goats.

Enzymes are protein catalysts present mostly in living cells and are constantly and rapidly degraded although, renewed by new synthesis (Coles, 1986). Zilva and Pannall (1984) stated that normal enzyme level in serum is a reflection of a balance between synthesis and their release, as a result of the different physiological processes in the body. Aspartate aminotransferase (AST) is an enzyme abundantly found in liver and heart muscles and plays an important role in amino acid metabolism (Vojta *et al.*, 2011). There was significant ($P < 0.05$) difference in AST levels among

the treatment groups and however fell within the normal range for apparent healthy goat as reported by Olaifa and Opara (2011). This result indicated the absence of necrosis, myocardial infarctions or hepatic metabolism; which are all indicators of good protein quality of the diets. This tends to suggest that the quality of protein in test diets were better than that of the control diet. The activities of the enzymes alanine transaminase (ALT) and alkaline phosphatase (ALP) studied were similar ($P>0.05$) among the treatment groups but however are within the reported reference range for goats. However ALT is an enzyme found in the highest amount in liver and typically used to detect liver injury (Pratt, 2010); thus indicating the safety of the diets for proper liver functioning. Guyton (1991) noted that ALP level in the blood is usually a good indicator of bone formation since osteoblasts secrete large quantities of this enzyme. The similarity of the ALP across the treatment could be said that the test diets did not adversely disrupt the activity of bone formation.

Table 4: Blood biochemistry of West African dwarf (WAD) goat fed provitamin A cassava peel-centrosema leaf meal based diets

Parameters	Dietary levels				SEM	Normal range
	A	B	C	D		
Total protein (g/l)	66.21 ^b	66.41 ^b	72.00 ^a	73.32 ^a	3.99	60 - 79 (g/l)
Albumin (g/l)	32.20 ^b	32.36 ^b	34.98 ^a	34.33 ^a	1.06	27 - 39 (g/l)
Globulin (g/l)	34.01 ^c	34.05 ^c	37.02 ^b	38.99 ^a	1.23	27 - 41(g/l)
Creatinine (mg/dl)	1.11 ^b	1.11 ^b	1.18 ^a	1.34 ^a	0.08	0.7 - 1.5 (mg/dl)
Urea (mg/dl)	17.85 ^a	15.52 ^b	16.21 ^b	13.11 ^c	0.39	10 - 27 (mg/dl)
Bilirubin (mg/dl)	0.18	0.20	0.19	0.22	0.02	0–1.71 (µmol/L)
Cholesterol (mg/dl)	62.71	63.07	61.40	62.32	3.04	40.1-127.3 (mg/dl)
AST (U/L)	27.12 ^a	24.74 ^b	20.91 ^c	19.61 ^c	7.87	12-38 (U/L)
ALT (U/L)	27.10	27.25	26.75	27.00	1.17	15.3-52.3 (U/L)
ALP (U/L)	81.25	82.75	81.75	82.50	4.26	61.3-283.3 (U/L)

a, b, c, d means in the row with different superscripts are significantly different ($P<0.05$)

AST = Aspartate aminotransferase

ALT = Alanine aminotransferase

ALP = Alkaline phosphatase

CONCLUSIONS

From this study, it could be concluded that provitamin A cassava peel-centrosema leaf meal based diets had no detrimental effect on haematological and serum biochemical parameters and therefore could be included in the diets of West African Dwarf goats up to 30%.

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Ethics approval and consent to participate

This paper followed all the guidelines for the care and use of laboratory animal model of the Federal College of Agriculture, Ishiagu, Ebonyi State, Nigeria.

Conflict of interest

The authors declare that they have no conflict of interest.

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PRELIMINARY RESULTS ON LIPID CONTENT OF SOYBEAN (*GLYCINE MAX* (L.) MERR.) AND RAPESEED (*BRASSICA NAPUS* L.) SEEDLINGS UNDER SALT STRESS

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Abstract: Seedlings of *Brassica napus* L. and *Glycine max* (L.) Merr. were investigated in terms of lipid accumulation, under moderate NaCl salt stress. No significant differences were recorded between control variants and salt-exposed seedlings when measured after four or seven days. Overall, soybean seedlings accumulated large amounts of lipids compared with those of rapeseed, despite that within soybean working variants, no consistent differences have been found.

INTRODUCTION

Rapeseed (*Brassica napus* L.) plants belong to the *Brassicaceae* (*Cruciferae*) family and represent the third most important edible oilseed crop source in the universe, after soybean (*Glycine max* (L.) Merr., *Fabaceae*) and palm (Ashraf and McNeilly 2004). Rapeseed, widely cultivated in several temperate regions, is major oil crop, accounting for more than 60 million tons of seed (Guo et al., 2014).

In plants, the lipid content response to abiotic factor is different depending on the species and class of lipids. Generally, in several species, the total lipid amount of leaves shows a decrease as a response to drought stress (Zhong et al., 2011). In mustard (*Brassica juncea*) seeds, total and neutral lipids declined considerably with increasing salt level, while phospholipids and glycolipids increased (Parti et al., 2003). Likewise, total lipid content in canola (*Brassica juncea* L.) was diminished with increasing NaCl levels (Keshtehgar et al., 2013). In addition, Bybordi (2011) found that the plasma membrane phospholipids were increased in some canola (*Brassica napus*) cultivars (*Elite*, *Licord*, and *Formax*) but did not change significantly in other (*SLM046* and *Okapi*) after exposure to NaCl salinity stress. Alqarawi et al. (2014) found that salt stress determined in *Ephedra alata* a significant decrease in total lipids, triacylglycerol and sterol and at the same time with an increase in diacylglycerol, sterol ester, and non-esterified fatty acids.

MDA is regarded as a marker for evaluation of lipid peroxidation or damage to plasmalemma and organelle membranes that increases with environmental stresses (Esfandiari et al., 2007). Generally, with the augmenting level of salt stress, the level of lipid peroxidation increased. For instance, within four investigated rapeseed cultivars, only in two of them an increase of lipid peroxidation has been reported (Farhoudi et al., 2015) On the other hands, lipid peroxidation was synchronized with increased of the salinity level which had a relation with plant species like tomato (Neumann, 2001), wheat (Hala et al., 2005), corn (Carrasco-Ríos and Pinto, 2014) etc. Furthermore, the fatty acid composition of the *Chlamydomonas mexicana* and *Scenedesmus obliquus* freshwater species was also improved by the increased NaCl concentration (Salama et al., 2013).

In order to continue the previous research (Oprica et al., 2011; Oprica, 2013) regarding the response of important economic plants to salt stress, the goal of this study was to determine the total lipid content in seedlings of two important edible oilseed *Brassica napus* L. cultivars (*Exagone* and *Exgold*) and *Glycine max* (L.) Merr.

MATERIAL AND METHODS

Soybean seeds necessary for the experiment were provided by the Laboratory of Microbiology, Faculty of Biology, Iasi. Rapeseed seeds were obtained from the Territorial Institute for Quality Seeds and Planting Material Iasi; two cultivars (*Exagone* and *Exgold*) of rapeseed were used in this study. Apparently, there are no data about salinity tolerance of these two cultivars. Firstly, 100 rape and soybean seeds were sterilized in 3% H₂O₂ and then rinsed to remove H₂O₂. These seeds were subjected to the treatment with 50mM, 100mM, and 150mM NaCl, by immersion in a correspondent salt solution for 4 hours. Control seeds for rape and soybean were stored for 4 hours in distilled water. Then the seeds were transferred into sterile Petri dishes, containing 2 layers of Whatman 1 paper, moistened with 10 ml of 50, 100, and 100 mM NaCl solutions and distilled water for the control sample. Each Petri dish contained 100 seeds and three repetitions for each working variant were further used. The Petri dishes were kept in the dark, at 25°C to promote the germination. After that, these were transferred to a room, assuring the normal conditions for seedling growth and watered with distilled water every

2 days (Oprica and Stefan, 2014). For lipid content determinations, the fresh seedlings were randomly sampled from each treatment at four and seven days, respectively after exposure to NaCl salt treatment.

Dry weight content was determined using a gravimetric method by the maintenance of the biological material at 105°C to constant weight. The results are expressed in g dry matter/100g fresh biological material (Boldor et al., 1983).

Lipid content. The content of total lipids was established using Soxhlet gravimetric methods, which consist in the extraction of the total lipids from dry weight of soybean and rapeseed seedlings, using petroleum ether. The total content of lipids was calculated depending on degreased samples and the results being expressed in g/100 g dried material (Artenie and Tanase, 1981).

All experiments were carried out with three independent repetitions. The results were expressed as the means value and standard errors of the mean.

RESULTS AND DISCUSSIONS

The effect of increasing of salinity stress on lipid content in *Brassica napus* cultivars and *Glycine max* seedlings of the two cultivars after 4 and 7 days after salinity treatment is shown in Figure 1. Generally, there are no significant differences between control and salt-exposed seedlings; this seems to be true for both moments of determination, at four and seven days, respectively. Increased salt stress seems not to induce alterations in lipid content of seedlings. Moreover, there are not differences between the two cultivars of rapeseed; only when exposed to 150 mM NaCl, *Exgold* cultivar seemed to accumulate a larger amount of lipids, a situation available only for the four-day determination.

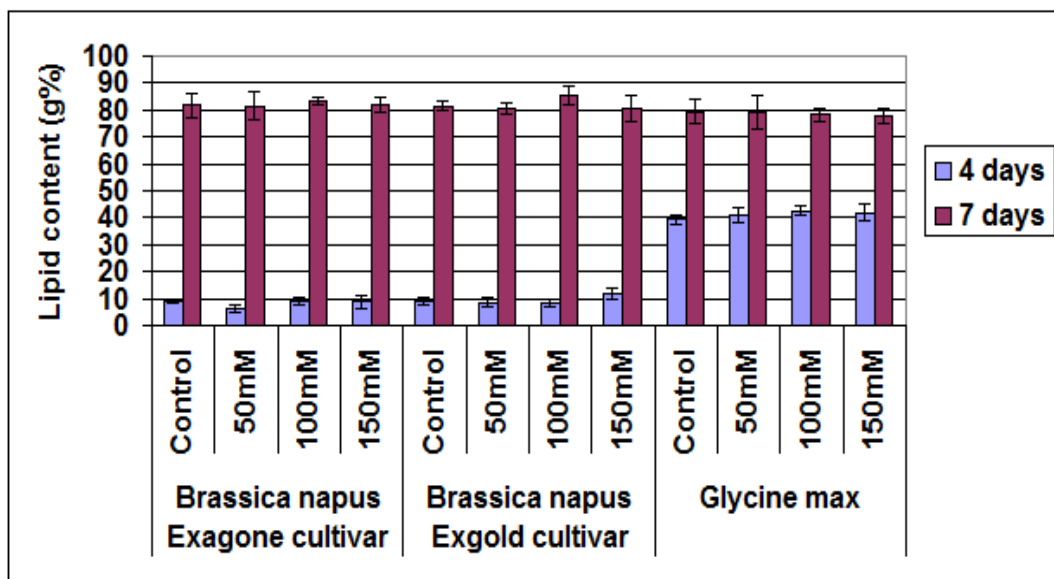


Figure 1. Lipid content in soybean and in two rapeseed cultivars (*Exagone* and *Exgold*)

However, the linear-like pattern of lipid accumulation measured after seven days in all analyzed samples may suggest either salt stress has not been so intense to induce noticeable differences or the time exposure was too short to enable the seedlings to build up responses in terms of lipid accumulation. It is well known that plants respond in different ways to salt stress,

depending on stress intensity, developmental stage, and laboratory work conditions (Grigore et al., 2011; 2012).

Soybean accumulated a larger amount of total lipids than both cultivars of rapeseed, but with no differences within the control and salt-exposed seedlings.

CONCLUSIONS

Our study revealed that there are no significant differences regarding total lipid accumulation in rapeseed and soybean seedlings exposed to NaCl salt stress. Nevertheless, prospective and extended studies will facilitate a deeper evaluation of the salinity tolerance of investigated cultivars/species, in order to achieve a better understanding of the general mechanisms involved in salt tolerance of highly valuable economic plants.

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**PROFESORUL UNIVERSITAR DR. VLAD
ARTENIE
LA A 80-A ANIVERSARE**

**PROFESSOR VLAD ARTENIE
AT THE 80-TH ANNIVERSARY**

Personalitate marcantă a biochimiei românești, creatorul școlii ieșene de biochimie, om de știință și dascăl de aleasă ținută intelectuală, profesorul Vlad Artenie se află astăzi în galeria marilor profesori ai Universității „Alexandru Ioan Cuza” din Iași.

Coleg de generație și de facultate cu profesorul octogenar, i-am cunoscut bine formarea și ascensiunea didactico-științifică, efortul de a crea o secție de Biochimie la Facultatea de Biologie a celei mai vechi universități din țară, munca permanentă de modernizare și diversificare a laboratoarelor de specialitate, de dotare a acestora, în care să lucreze deopotrivă studenți, masteranzi și doctoranzi, sub conducerea cadrelor didactice selectate cu grijă și promovate corect de sârbătoritul nostru.

De-a lungul timpului i s-au dedicat de către specialiști în domeniu, medalioane aniversare la împlinirea vârstei de 75 de ani, în care este prezentată pe larg viața și activitatea desfășurată pe parcursul celor peste cinci decenii de carieră didactică și de cercetare științifică.

Scurt periplu biografic

Vlad Artenie a văzut lumina zilei pe 16 septembrie 1936, în satul Valea Glodului, comuna Vulturești (Județul Suceava), din părinții Catinca și Grigore Artenie (mama – țărancă/agricultoare și tata – tractorist/mecanic agricol), oameni simpli, gospodari și cinstiți de pe meleagurile Bucovinei, dornici de a-și vedea fiul plecând la școli înalte. Urmează școala primară și gimnazială (1944 -1951) în localitatea natală, iar apoi este elev la Liceul „Nicu Gane” din Fălticeni (în perioada 1951 - 1954). După susținerea cu succes a examenului de bacalaureat, în anul 1954 devine student al Facultății de Chimie (secția Chimie organică) de la Universitatea „Alexandru Ioan Cuza” din Iași, pe care o va absolvi cu rezultate deosebite în anul 1959. În al său curriculum vitae scrie cu mândrie că a avut ca dascăli pe renumiții profesori Radu Cernătescu, Margareta Poni, Ioan Golovcenco, Florica Câmpan, Elena Budeanu, Magda Petrovanu, Ioan Zugrăvescu, Eugen Papafil, Constantin Budeanu, Boris Arventiev, Vasile Ababii (acesta din urmă conducându-i lucrarea de licență intitulată „Preparate chimico-terapice pe bază de arsenic, stibiu și bismut”), iar ca șefi de

An outstanding personality of the Romanian biochemistry, the creator of the Biochemistry School of Iasi, a scientist and a teacher of high intellectual attire, Professor Vlad Artenie has earned his place in the gallery of the great professors of the „Alexandru Ioan Cuza” University of Iași.

Being part of the same generation as well as a faculty colleague with the octogenarian professor, I am well acquainted with his training and didactic-scientific ascension, and his efforts of creating a specialization(section) of Biochemistry within the Faculty of Biology of the country’s most ancient university, the continuous work he has put into the modernization and diversification of the biochemistry laboratories and their equipment, creating the environment for undergraduate students, master and PhD students to develop, under the guidance of the teaching staff, which was carefully selected and correctly promoted by our feted colleague.

Over time, the specialists in the field have dedicated to Professor Artenie anniversary medallions at the age of 75, occasion with each the life and activity spanning over more than five decades of didactic career and scientific research had been presented at length.

Brief biographical periplus

Vlad Artenie saw the light of day on September 16th, 1936, in the village of Valea Glodului, Vulturești commune (Suceava County). His parents, Catinca and Grigore Artenie (the mother – a peasant woman and the father – a tractor driver), were simple, hard-working and honest persons from Bucovina lands, eager to see their son departing to high schools. He attends the primary and secondary school (1944 -1951) in his home locality and then he continues his studies at the „Nicu Gane” High school in Fălticeni (over the period from 1951 to 1954). After he successfully passed the baccalaureate examination, in 1954 he is admitted to the Faculty of Chemistry (the Organic Chemistry speciality), „Alexandru Ioan Cuza” University of Iași, from where he graduates with remarkable results in 1959. In his curriculum vitae, he proudly writes that his teachers were the prestigious Prof. Radu Cernătescu, Margareta Poni, Ioan Golovcenco, Florica Câmpan, Elena Budeanu,

lucrări sau asistenți, pe Iulian Zaharia, Emilia Gherasimescu, Valeriu Pop, Lidia Dascălu, Eugenia Rucinschi, Domnica Furnică, Ana Kleinștein, Natalia Hurduc, Leonte Candiano, Angela Popa și alții.

Fiind și eu student în acei ani la Facultatea de Științe Naturale, am cunoscut pe unii din chimiștii menționați, câțiva fiindu-mi și mie cadre didactice, iar la unele cursuri l-am cunoscut și pe colegul Vlad Artenie, cel care din 1974 a devenit conferențiar la Facultatea de Biologie-Geografie-Geologie, lucrând împreună de 42 de ani, audiindu-ne reciproc cursurile, conducând amândoi o vreme destinele facultății, făcând parte din Consiliul Național de Evaluare Academică și de Acreditare a instituțiilor de învățământ superior din România.

Pentru rezultatele de excepție obținute în anii studenției și la examenul de licență, proaspătul absolvent a fost numit, în 1959, preparator la disciplina Biochimie, având-o ca îndrumător pe conferențiarul Eliza Văscăuțeanu. În anul Centenarului Universității noastre (1960) a fost promovat prin concurs la gradul de asistent, funcție în care va lucra până în 1967. În anul 1963 obține o bursă din partea Ministerului Învățământului pentru a se califica prin doctorat la renumita Universitate de Stat „M. V. Lomonosov” din Moscova, avându-l conducător științific pe academicianul Serghei E. Severin (reputat biochimist al aceluși timp). În anul 1966, colegul Vlad Artenie își susține teza de doctorat în științe biologice, specialitatea biochimie animală, cu subiectul „Izolarea, purificarea și proprietățile colinacetilazei”.

În anul 1966 revine în țară, iar în 1967 este promovat, prin concurs, șef de lucrări la aceeași disciplină de biochimie, în care-și susținuse teza de doctorat, apoi în 1969 devine conferențiar la Facultatea de Chimie pe care o absolvise în urmă cu zece ani.

Din 1974 până în prezent este cadru didactic la Facultatea de Biologie : conferențiar până în 1990, profesor titular din 1990 și profesor consultant din 2005.

Activitatea didactică desfășurată în calitate de cadru didactic, activ și consultant, pe parcursul a peste cinci decenii, este prodigioasă, susținută de 3 îndrumătoare de laborator, 3 prelegeri de sinteză, 4 cursuri (de chimie organică și de chimie biologică) și 3 manuale, la toate fiind unic sau prim autor, una dintre cărți, publicată într-o editură centrală, având caracter monografic. Toate cele 13 materiale (monografii, cursuri, îndrumătoare de lucrări

Magda Petrovanu, Ioan Zugrăvescu, Eugen Papafil, Constantin Budeanu, Boris Arventiev, Vasile Ababii [the latter supervising his diploma paper(licence thesis) entitled „Chemico-therapeutic Preparations Based on Arsenic, Antimony and Bismuth”], and his assistant professors or junior lecturers were Iulian Zaharia, Emilia Gherasimescu, Valeriu Pop, Lidia Dascălu, Eugenia Rucinschi, Domnica Furnică, Ana Kleinștein, Natalia Hurduc, Leonte Candiano, Angela Popa and others.

Being a student myself in those years at the Faculty of Natural Sciences, I have known some of the chemists mentioned above, some of which had been my teachers too, and in certain courses I met our colleague Vlad Artenie, and since 1974 he has been an Associate Professor at the Faculty of Biology-Geography-Geology, where, we have worked together for 42 years, mutually hearing our courses, being in charge for a while of the faculty destiny, being members in the National Council of Academic Assessment and Accreditation of the higher education institutions in Romania.

In 1959, by virtue of the exceptional results obtained in the student years and at the final university examination, the fresh graduate was appointed research assistant in Biochemistry discipline, under the tutorship of Associate Professor Eliza Văscăuțeanu. In the Centenary year of our University (1960), he was appointed assistant lecturer in the process of tenure track evaluation, position that he keeps until 1967. In 1963, he obtains from the Ministry of Education a bursary for PhD studies at the well-known „M. V. Lomonosov” State University of Moscow, where he had as supervisor the academician Serghei E. Severin (biochemist of good repute at that time). In 1966, the colleague Vlad Artenie defends his PhD thesis in Biological Sciences, specialty – Animal Biochemistry, on the topic of „Isolation, Purification and Properties of the Choline Acetylase”.

In 1966, he returns to the country and in 1967, he is promoted, through tenure track evaluation, to Assistant Professor(lecturer), in the same discipline of Biochemistry, in which he had defended his PhD thesis. Then, in 1969, he becomes an Associate Professor at the Faculty of Chemistry, from where he had graduated ten years before.

From 1974 to date, he is a member of the teaching staff at the Faculty of Biology: Associate Professor until 1990, tenured Professor from 1990 onwards and Consulting Professor from 2005.

practice) se adresează deopotrivă studenților, masteranzilor, doctoranzilor și tinerilor cercetători, dar și specialiștilor din diferite domenii (biologic, medical, agronomic), care necesită cunoștințe de biochimie generală (vegetală și animală) sau capitole speciale, precum metabolismul lipidelor, biosinteza acizilor nucleici și a proteinelor, acizii nucleici și rolul lor genetic ș.a. Toate materialele didactice publicate sunt bine fundamentate teoretic, periodic actualizate, prezentate cu o claritate de cristal, ca și prelegerile prezentate în laborator sau amfiteatru. În îndelungata sa activitate didactică a condus un număr impresionant de lucrări de licență (259) și de disertație (44), precum și lucrări științifico-metodice pentru obținerea gradului didactic I în învățământul preuniversitar (6), fiind totodată președinte la multe (55) comisii pentru acordarea acestui titlu. Exigent cu sine însuși în primul rând, era exigent, dar corect, în evaluarea studenților, masteranzilor, doctoranzilor și profesorilor din învățământul preuniversitar, care totdeauna apreciau pe profesor nu numai pentru modul cum preda cursurile, dar și pentru răbdarea și înțelepciunea manifestate în sesiunile de examen.

Pentru studenți și doctoranzi era prezent zilnic în facultate, de dimineața până seara târziu ; se interesa de dotarea laboratoarelor cu aparatură și substanțe chimice, cu care se întorcea de fiecare dată când pleca la specializare și documentare în străinătate. Amintește adesea de colegii de la universitățile care l-au sprijinit pe parcursul anilor : prof.univ.dr. Roderich Brandsch (Germania), prof. univ. dr. Jean Montreuil, prof. univ. dr. Didier Guillochon și director de cercetare II dr. Rita Barot (Franța), prof. univ. dr. Franca Majone (Italia), prof. univ. dr. P. D. Grigorcea și V. Ciobanu (Chișinău, R. Moldova).

Profesorul Vlad Arteni a avut o contribuție importantă la înființarea modului franco-român pentru masteratul de „Enzimologie și Biotehnologie” (Facultatea de Chimie), precum și la fondarea și implementarea masteratului franco-român „Bio-procedee în domeniul agroalimentar” (Facultatea de Biologie).

Activitatea științifică, foarte bogată și unanim apreciată în cercurile de specialiști, s-a desfășurat în următoarele domenii : Enzimologie teoretică și aplicată, Biochimia proteinelor, Biochimie animală, vegetală și ecologică, Biochimie clinică, Biochimia microorganismelor, Biologie moleculară.

The didactic activity carried out as an active and Consulting Professor, over more than five decades, is prodigious and it consists, among others, of 3 laboratory guides, 3 synthesis lectures, 4 courses (in organic chemistry and biological chemistry) and 3 handbooks for which he was the unique or first author. One of the books was published in a central publishing house and has a monographic nature. All these 13 materials (monographs, courses, practical works guides) are addressed at the same time to undergraduate students, master and PhD students and to young researchers, but also to specialists in various fields (of biology, medicine, agronomy) that require knowledge of general (vegetal and animal) biochemistry or special chapters, such as lipid metabolism, biosynthesis of the nucleic acids and of proteins, nucleic acids and their genetic role etc. All the didactic materials published are well grounded from a theoretical point of view, have been periodically updated and have been presented with a crystal clarity, such are also his lectures presented in the laboratory or in the amphitheatre. During his long didactic activity, he supervised an impressive number of undergraduate (259) and dissertation (44) theses, as well as scientific-methodical works for obtaining the level I didactical qualification in the pre-university education (6), being at the same time chairman in several (55) commissions for the award of this title. Strict with himself first of all, the Professor was demanding, but fair, in assessing the students, the master students and PhD candidates and the teachers in the pre-university education, who have always appreciated him, not only for the manner in which he taught the courses, but also for the patience and wisdom displayed during the exam sessions.

For his students and PhD candidates, he was present every day in the faculty, from morning until late at night; he showed interested in the endowment of the laboratories with new devices and chemical substances, that he used to bring each time when he went abroad for specialization and documentation purposes. He often remembers the colleagues from other universities who have supported him over the years: Prof. Roderich Brandsch (Germany), Prof. Jean Montreuil, Prof. Didier Guillochon and Research Director II Rita Barot (France), Prof. Franca Majone (Italy), Prof. P. D. Grigorcea and V. Ciobanu (Chișinău, Republic of Moldova).

Professor Vlad Arteni had brought important contribution to the foundation of the French-Romanian module for the master program of

Profesorul Vlad Artenie își începe ucenicia sub bagheta marilor profesori de la Facultatea de Chimie, al cărei student a fost, și pentru primele rezultate publicate împreună cu aceștia este recomandat și pleacă, în 1963, să-și facă teza de doctorat în fosta Uniune Sovietică, la renumita Universitate „Lomonosov” din Moscova. Întors în țară, din 1966 continuă munca de cercetare în laboratoarele Facultăților de Chimie (până în 1974) și de Biologie (din 1974 până în prezent), mai întâi împreună cu seniorii din domeniu, după care se desprinde și-și formează propria echipă, care se va mări treptat odată cu înființarea, de către colegul Vlad Artenie, a secției de Biochimie de la Facultatea de Biologie.

Nu am căderea să fac referiri detaliate asupra operei științifice a profesorului Vlad Artenie. Au făcut-o alții înaintea mea, specialiști în domeniu, cu prilejul aniversării celor 75 de ani (în 2011) a colegului meu, ori atunci când i s-a acordat (în 2010) premiul OPERA OMNIA de către Consiliul Național al Cercetării Științifice din Învățământul Superior.

Bogata sa activitate de cercetare științifică s-a materializat într-un număr impresionant de studii, comunicări și articole originale, toate din domeniul miraculos al Biochimiei: 98 (29 în extenso și 69 în rezumat) publicate în reviste de specialitate din străinătate și 276 (217 în extenso și 59 în rezumat) publicate în reviste din țară, la cele mai multe fiind prim autor. Înainte de a fi publicate, rezultatele cercetării au fost comunicate la manifestări științifice din țară (57) și din străinătate (46). La acestea se adaugă 9 brevete de invenție, 10 lucrări din istoria biologiei și chimiei, 30 recenzii; o lungă perioadă de timp a lucrat la granturi de cercetare, în unele fiind coordonator (19, cu finanțare internă), la altele colaborator (3 cu finanțare internă și 1 cu finanțare externă). În același context subliniem contribuția colegului Vlad Artenie ca expert al C.N.C.S.I.S. și ca director al Centrului de Biochimia și Genetica Celulelor Microbiene și Vegetale.

În perioada 1982 – 1990 conduce cu delegație doctorat în specialitatea Biochimie, iar din 1990 este conducător științific titular până în prezent. Este de subliniat numărul mare (67) de doctori în științe biologice, specialitatea biochimie, formați de profesorul Vlad Artenie, din care 8 în cotutelă cu universități din Franța și Republica Moldova și 3 din țări străine (Grecia, Israel, Ucraina). Totodată, profesorul Vlad Artenie a fost membru în mai multe

„Enzymology and Biotechnology” (Faculty of Chemistry), but also to the foundation and implementation of the French-Romanian master program “Bio-procedures in the Agro-alimentary field” (Faculty of Biology).

His scientific activity, very rich and unanimously appreciated in the specialists’ circles, was carried out in the following fields: Theoretical and Applied Enzymology, Biochemistry of Proteins, Animal, Vegetal and Ecological Biochemistry, Clinical Biochemistry, Biochemistry of Microorganisms, Molecular Biology.

Professor Vlad Artenie starts his training under the supervision of the great professors of the Faculty of Chemistry, where he did his undergraduate studies, and for the first results published together with them he is recommended for a PhD bursary, and, in 1963, he goes to elaborate his PhD thesis in ex-Soviet Union, at the prestigious „M. V. Lomonosov” University of Moscow. Returning to the country afterwards, from 1966 he continues the research work in the laboratories of the Faculty of Chemistry (up to 1974) and then the Faculty of Biology (from 1974 to date), initially working together with the senior figures in the field, he then later separates and forms his own research team, which shall gradually increase once with the foundation by the colleague Vlad Artenie of the Biochemistry section within the Faculty of Biology.

I am not going to make detailed references to the scientific creation of Professor Vlad Artenie. Others, specialists in the field, have done it before me, with the occasion of the 75 years anniversary (in 2011) of my colleague, or when he received (in 2010) the OPERA OMNIA award from the National Council of Scientific Research in Higher Education.

His rich scientific research activity has materialized in an impressive number of original studies, communications and articles, all from the marvelous field of Biochemistry: 98 (29 in extenso and 69 in abstract) published in foreign specialty magazines and 276 (217 in extenso and 59 in abstract) published in Romanian periodicals, with Professor Artenie being the first author of most of them. Before being published, the results of his research have been communicated in many scientific manifestations in the country (57) and abroad (46). We can also add 9 patents, 10 works about the history of biology and chemistry, 30 reviews; he had worked for a long period of time in research grants, in some of them as a coordinator (19, with internal financing), in others as a

(107) comisii de doctorat, inclusiv 7 din Republica Moldova.

Pentru rezultate deosebite în studiul unor microorganisme producătoare de substanțe biologice active, în anul 1977 a fost distins cu premiul „Emanoil Teodorescu” al Academiei Române. În anul 1988 i se acordă titlul de „Conferențiar universitar evidențiat” de către Ministerul Învățământului, iar în anul 2007 i se acordă titlul de „Profesor emeritus” de către Senatul Universității „Alexandru Ioan Cuza” din Iași.

Prin competența-i cunoscută și recunoscută, a fost referent științific la 32 cărți publicate în diferite edituri din țară și membru în comisii de promovare a unor cadre didactice și cercetători din Iași și din alte centre universitare.

După specializarea prin doctorat la Universitatea „Lomonosov” din Moscova (1963 - 1966), în perioada 1993 - 1997 efectuează scurte stagii de documentare și specializare (în cadrul Proiectului TEMPUS – PEC) în Franța (Paris, Lille), Belgia (Leuven) și Italia (Padova), iar în perioada 2000 – 2006 este invitat de mai multe ori la universități din Germania (Freiburg) și Franța (Lille), de unde totdeauna s-a întors cu literatură de specialitate, aparate și substanțe pentru cele 4 laboratoare de biochimie organizate în Facultatea de Biologie.

Profesorului Vlad Artenie i se datorește organizarea unor manifestări științifice naționale (12) și internaționale, în perioada 1995 -2004 fiind director – din partea Universității „Alexandru Ioan Cuza” din Iași – a Școlii de Vară Franco-Română de Biochimie, organizată în colaborare cu Universitatea de Științe și Tehnologii din Lille (avându-l ca director pe profesorul Jean Montreuil, membru de onoare al Academiei Române, Doctor Honoris Causa al Universității „Alexandru Ioan Cuza”).

Parte din timp este rezervată activității editoriale, biochimistul Vlad Artenie fiind membru în comitete de redacție ale unor reviste naționale, locale (Analele științifice ale Universității „Alexandru Ioan Cuza” din Iași, seria II, secțiunea „Genetică și Biologie Moleculară”, al cărui redactor șef este) și centrale (Studii și cercetări de biochimie, Revue roumaine de biochimie, Romanian Journal of Physiology-Physiological Sciences), dar și din străinătate (Comisia de Cercetare Consultativă și de Recomandare Științifică a Institutului Biografic American, din SUA).

collaborator (3 with internal financing and 1 with external financing). In the same context, we highlight the contribution of colleague Vlad Artenie as an expert of C.N.C.S.I.S. and as a director of the Center of Biochemistry and Genetics of the Microbial and Vegetal Cells.

Over the period 1982 – 1990, he administers by delegation the PhD program in the Biochemistry specialty and from 1990 to date he has been a tenured scientific coordinator. We must highlight the large number (67) of doctors in biological sciences, the specialty of biochemistry, formed by Professor Vlad Artenie, of whom 8 in collaboration with the universities of France and the Republic of Moldova and 3 from foreign countries (Greece, Israel, Ukraine). Moreover, Professor Vlad Artenie was a member in several (107) PhD examination boards, including 7 in the Republic of Moldova.

For the impressive results in the study of certain microorganisms producing active biological substances, he received in 1977 the „Emanoil Teodorescu” award of the Romanian Academy. In 1988, the Ministry of Education awards him the title of „Distinguished Associate Professor” and in 2007 the Senate of the „Alexandru Ioan Cuza” University of Iași grants him the title of „Professor Emeritus”.

By his well-known and recognized competence, he has been the scientific supervisor of 32 books published by different publishing houses in the country, and a member in commissions of promotion of certain members of the didactic staff of Iași and of other university centers.

After the PhD specialization at the „Lomonosov” University of Moscow (1963-1966), over the period 1993 – 1997, he performs short documentation and specialization traineeships (within the TEMPUS – PEC Project) in France (Paris, Lille), Belgium (Leuven) and Italy (Padua). Over the period 2000 – 2006 he is invited several times at the universities of Germany (Freiburg) and France (Lille), from where he always brought back specialty literature, devices and substances for the 4 biochemistry laboratories organized within the Faculty of Biology.

Professor Vlad Artenie was in charge of the organization of certain national (12) and international scientific manifestations, over the period 1995-2004, being the Director – on the part of „Alexandru Ioan Cuza” University of Iași – of the Franco-Romanian Summer School of Biochemistry, organized in collaboration with the University of Sciences and Technologies of Lille (whose Director was Professor Jean Montreuil, member of honor of

Activitatea managerială constă în activitățile desfășurate ca secretar științific al Consiliului profesoral al Facultății de Biologie și membru în Consiliul profesoral al Facultății de Chimie, ca șef de Catedră (1991 – 1995, 1998 – 2005) și membru ales în Consiliul Național de Evaluare Academică și Acreditare (1994 – 2000).

Pentru apreciată activitate de cercetare științifică, profesorul Vlad Artenie a fost ales membru al unor societăți de prestigiu din România – Societatea de Științe Biologice, Societatea de Biochimie și Biologie Moleculară (fiind președintele Filialei Iași), Asociația Oamenilor de Știință (Filiala Iași), Fundația MEDBIOCHIM București, Fundația Internațională „Ștefan Lupașcu” pentru Științe și Cultură (Filiala Iași).

Date biografice despre profesorul Vlad Artenie sunt incluse în „Dicționarul Biologilor Români” (Arad, 2000), Dicționarul specialiștilor „Who’s Who” în România (București, 2002), Enciclopedia Bucovinei (Suceava, 2005), Cartea Fălticeniilor – de la A la Z (Iași, 2005), Enciclopedia Personalităților din România, Hübners Who is Who, București, 2010 și Dicționarul personalităților din România: biografii contemporane/ONG ECO-Europa, Editura Anima, București, 2013.

Despre activitatea didactică, științifică și managerială au scris, în 2011, profesorul Savel Ifrim (Contribuții ieșene în știința chimică – Editura Performantica, Iași) și profesorul Dumitru Cojocar (Analele Științifice ale Universității „Alexandru Ioan Cuza” din Iași, Secțiunea II, Genetică și Biologie Moleculară, Tomul XII, Fascicula 3).

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Îl cunosc, așadar, pe domnul profesor emerit Vlad Artenie de când am devenit studenți ai Almei Mater Iassensis, eu la Facultatea de Biologie (în 1953), el la Facultatea de Chimie (în 1954). Am deveni colegi în 1974, când Colectivul de Chimie și Biochimie, condus de ilustrul meu coleg, s-a integrat în Facultatea de Biologie. De peste patru decenii îi cunosc activitatea profesională, îndelungata experiență didactică, științifică și organizatorică, efortul de a înființa și conduce noua secție de Biochimie (înființată în anul 1991), de a dota laboratoarele cu aparatură și substanțe necesare dezvoltării domeniului pe care îl slujește de aproape șase decenii.

the Romanian Academy, Doctor Honoris Causa of the „Alexandru Ioan Cuza” University).

Part of his time he devoted to the editorial activity, biochemist Vlad Artenie being a member of the editorial committees of certain national, local (Scientific Annals of the „Alexandru Ioan Cuza” University of Iași, series II, section „Molecular Genetics and Biology”, being its editor-in-chief) and central magazines (Biochemistry Studies and Research, *Revue roumaine de biochimie*, Romanian Journal of Physiology-Physiological Sciences), as well as some foreign ones (Committee of Consultative Research and Scientific Recommendation of the American Biographic Institute of the USA).

His managerial activity consists in the activities carried out as a scientific secretary of the Teachers’ Council of the Faculty of Biology and as a member in the Teachers’ Council of the Faculty of Chemistry, as Head of Chair (1991 – 1995, 1998 – 2005) and as an elected member in the National Council for Academic Assessment and Accreditation (1994 – 2000).

For his appreciated scientific research activity, professor Vlad Artenie was elected as the member of certain prestigious societies in Romania – the Society of Biological Sciences, the Society of Biochemistry and Molecular Biology (being the Chairman of the Subsidiary of Iași), the Association of Scientists (Subsidiary of Iași), the MEDBIOCHIM Foundation (Bucharest), the „Ștefan Lupașcu” International Foundation for Sciences and Culture (Subsidiary of Iași).

Biographical data on Professor Vlad Artenie are included in the *Dicționarul Biologilor Români* (Arad, 2000), “Who’s Who” Specialists’ Dictionary in Romania (Bucharest, 2002), *Enciclopedia Bucovinei* (Suceava, 2005), *Cartea Fălticeniilor – de la A la Z* (Iași, 2005), *Enciclopedia Personalităților din România*, Hübners Who is Who, Bucharest, 2010 and *Dicționarul personalităților din România: biografii contemporane /ONG ECO-Europa*, Anima Publishing House, Bucharest, 2013.

Professor Savel Ifrim (*Contribuții ieșene în știința chimică* – Performantica Publishing House, Iași) and Professor Dumitru Cojocar (Scientific Annals of the „Alexandru Ioan Cuza” University of Iași, Section II, Molecular Genetics and Biology, Tome XII, Fascicle 3) wrote in 2011 on his didactic, scientific and managerial activity.

Am lucrat împreună la fundamentarea planurilor de învățământ și am predat amândoi la cele trei secții ale facultății, am fost împreună în comisii de licență, disertație și doctorat, am contribuit împreună, ca șefi de Catedră și membri ai Consiliului profesoral, la bunul mers al facultății noastre, am făcut parte, în același timp, din Consiliul Național de Evaluare Academică și Acreditare.

Colegul Vlad Artenie, om așezat, serios, responsabil și echilibrat, onorează Facultatea și Universitatea în care a lucrat și lucrează, neprecupețind nici un efort pentru formarea, selectarea și promovarea corectă de viitori biochimisti. Este, astăzi, unul din cei mai mari biochimisti români, apreciat deopotrivă în țară și peste hotare pentru valoroasa operă științifică clădită împreună cu colaboratorii pe care i-a format.

Profesorul Vlad Artenie este o fire blândă, modestă, plină de bunătate și generozitate, mereu disponibil să ajute, să sprijine pe tineri în mod deosebit. Acesta este omul, iubit de colegi și studenți, prietenos, înțelept și bun sfătuitor, model de dascăl, cercetător și organizator. Acesta este omul patriot, un adevărat român. Prin toate alesele calități intelectuale și umane, profesorul Vlad Artenie și-a câștigat prețuirea și respectul tuturor celor care l-au cunoscut și îl cunosc : om integru, corect și onest !

La împlinirea venerabilei vârste de 80 de ani vă doresc, domnule profesor, multă sănătate și liniște sufletască.

„La mulți ani”, stimat coleg!

Academician Constantin TOMA
Facultatea de Biologie, Universitatea „Alexandru
Ioan Cuza”, Iași, ROMANIA

Therefore, I have known Professor Emeritus Vlad Artenie since we became students of Alma Mater Iassensis, I at the Faculty of Biology (in 1953), at the Faculty of Chemistry (in 1954). We became colleagues in 1974, when the Collective of Chemistry and Biochemistry, directed by my illustrious colleague, was integrated in the Faculty of Biology. I have known since more than four decades his professional activity, his wide didactic, scientific and organizational experience, his effort of establishing and managing the new section of Biochemistry (established in 1991), of equipping the laboratories with the devices and substances needed for the development of the field that he has served for almost six decades.

We worked together in the foundation of the curricula and we taught together at the three sections of the faculty, we were together in BA, MA and PhD examination boards, we have contributed together, as Heads of the Chair and member of the Teachers' Council to the good development of our faculty, we were part, at the same time, of the National Council for Academic Assessment and Accreditation.

The colleague Vlad Artenie, a wise, earnest, responsible and balanced person honors the Faculty and the University where he has been working, sparing no efforts for the correct training, selection and promotion of the future biochemists. Nowadays, he is one of the greatest Romanian biochemists, appreciated both in the country and overseas for the valuable scientific creation built together with the collaborators that he had trained.

Professor Vlad Artenie is a gentle, modest, kind and generous nature, always available to help, to support mainly young people. This is the friendly, wise man loved by his colleagues and students, a good adviser, an exemplar teacher, a researcher and an organizer. This is the patriot man, a true Romanian. By all his distinguished intellectual and human qualities, Professor Vlad Artenie had earned the appreciation and the respect of all those who have known him: an upright, fair and honest person!

At the anniversary of the venerable age of 80, I wish you, dear Professor, good health and peace of mind.

“Happy birthday”, esteemed colleague!

Academician Constantin TOMA
Faculty of Biology, „Alexandru Ioan Cuza”
University, Iași, ROMANIA

Lăcrămioara Oprică: METABOLIȚI SECUNDARI DIN PLANTE. ORIGINE, STRUCTURĂ, FUNCȚII, Editura Universității „Alexandru Ioan Cuza” Iași, 2016, 294 pagini, ISBN 978-606-714-253-2.

Lăcrămioara Oprică : SECONDARY METABOLITES OF PLANTS. ORIGIN, STRUCTURE, FUNCTIONS, „Alexandru Ioan Cuza” University Publishing House, Iași, 2016, 294 pages, ISBN 978-606-714-253-2.

La începutul anului 2016, în Editura **Universității „Alexandru Ioan Cuza”**, a apărut cartea intitulată **METABOLIȚI SECUNDARI DIN PLANTE. ORIGINE, STRUCTURĂ, FUNCȚII**, a cărei autoare este doamna doctor în biologie (specialitatea Biochimie) Lăcrămioara Oprică, lector în cadrul Laboratorului de Biochimie și Biologie Moleculară de la Facultatea de Biologie a Universității „Alexandru Ioan Cuza” din Iași, România. Apreciam că este vorba despre o carte remarcabilă care tratează o gamă largă de aspecte diferite privind metaboliții secundari sintetizați de plante, în nișa lor ecologică.

Această monografie are 294 de pagini și este structurată judicios în 7 capitole bogat ilustrate cu 113 figuri și 43 de tabele care redau formule structurale, reacții chimice, diagrame ale căilor de biosinteză, clasificări și acțiuni fiziologice ale metaboliților secundari etc. În elaborarea cărții autoarea a reușit să îmbine armonios rezultatele cercetărilor științifice proprii cu bogatele date informative din literatura de specialitate.

Concret, cartea detaliază, pe lângă diversele generalități privind compușii organici din organismele vegetale, particularitățile principalelor clase de metaboliți secundari (compuși fenolici, glicozide, terpeni, alcaloizi, uleiuri esențiale, rășini și balsamuri), cu referiri la clasificarea, structura chimică, biosinteza, funcțiile fiziologice și utilizările practice ale acestor adevărate „secrete” posedate de majoritatea speciilor de plante, menționându-se numeroși reprezentanți cu influențe asupra sănătății omului.

Primul capitol, intitulat *Generalități privind compușii naturali din plante* prezintă considerații generale utile referitoare la clasificarea, biosinteza și funcțiile metaboliților secundari. Autoarea are meritul că a încercat și a reușit să ofere o actualizare a funcțiilor exercitate de metaboliții secundari din plante, aducând clarificări asupra modurilor lor de acțiune, precum și în ce privește utilizarea acestor substanțe organice în farmacologie și medicină ca

At the beginning of 2016, at University Publishing House "Alexandru Ioan Cuza", appeared the book called **SECONDARY METABOLITES OF PLANTS. ORIGIN, STRUCTURE, FUNCTION**, whose author is the doctor in biology (specialization Biochemistry) Lăcrămioara Oprică, lecturer in the Laboratory of Biochemistry and Molecular Biology at the Biology Faculty of the "Alexandru Ioan Cuza" University from Iasi, Romania. We appreciate that it is a remarkable book which treats a wide range of different issues on secondary metabolites synthesized by plants in their ecological niche.

This monograph has 294 pages and is judiciously divided into 7 chapters, richly illustrated with 113 figures and 43 tables which give structural formulas, chemical reactions, diagrams of biosynthetic pathways, classifications and physiological actions of secondary metabolites etc. In elaborating of the book the author has managed to harmoniously combine own scientific research results with rich informative data from the literature.

Concretely, the book details, in addition to various generalities on organic compounds from vegetable organisms particularities of the main secondary metabolites classes (phenolic glycosides, terpenes, alkaloids, essential oils, resins and balsams) with reference to the classification, chemical structure, biosynthesis, physiological functions and practical uses of these true "secret" owned by most plants species, specifying the many compound with influences on human health.

The first chapter, entitled *Generalities regarding plant natural compounds* presents useful general considerations relating to the classification, functions and biosynthesis of secondary metabolites. The author has the merit that tried and failed to provide an update of the functions performed by secondary metabolites from plants, bringing clarification on their action modes, and the use of these organic substances in pharmacology

agenți terapeutici, în agricultură ca pesticide, precum și în alte domenii.

Următorul capitol, **al doilea**, se referă la compușii fenolici care reprezintă un grup mare de metaboliți secundari, cuprinzând atât fenoli simpli și derivați ai acestora, cât și polifenoli, cu diferite funcții în creșterea, dezvoltarea și apărarea plantelor. Autoarea menționează diverse exemple de fenoli simpli, precum pigmenți și arome care pot atrage sau respinge polenizatorii plantelor cu flori. De asemenea, se acordă o atenție deosebită metaboliților secundari ce pot proteja plantele împotriva insectelor, fungilor, bacteriilor și virusurilor. Mai mult, în acest capitol, putem găsi date despre structura chimică a componentilor precursori principali ai ligninelor, polimeri fenilpropanici ce formează elementele constitutive importante ale pereților celulelor din țesuturile conducătoare specifice plantelor. Acest capitol se încheie cu un subcapitol în care se discută structurile chimice variate ale taninurilor condensate și hidrolizabile, care datorită capacității lor de a lega proteinele protejează plantele de a fi mâncate de animale, având și alte funcții fiziologice în viața plantelor. Nu trebuie să uităm numeroasele utilizări practice ale taninurilor.

Capitolul 3 este consacrat glicozidelor, compuși chimici larg răspândiți în numeroase familii de plante. Utilizate atât în medicina tradițională cât și în medicina modernă, glicozidele exercită un spectru larg de acțiuni terapeutice, variind de la cardiotonice, analgezice, antiinflamatoare și anti-reumatice etc.

Capitolul 4 redă informații referitoare la terpene (terpenoide), compuși organici naturali formați din una sau mai multe unități de isopren (2-metil-1,3-butadienă), de unde și denumirea de isoprenoide. Aceste substanțe, cunoscute încă din cele mai vechi timpuri, sunt răspândite în plante sub formă de compuși biologic activi de o largă varietate și se bucură de utilizări practice diverse ca medicamente, arome, coloranți, parfumuri, substanțe chimice folosite în agricultură etc.

Capitolul 5 se concentrează pe aspecte esențiale privind alcaloizii, o familie mare de metaboliți secundari care conțin în structura lor atomul de azot și care se găsesc în peste 1200 de specii de plante. Se subliniază faptul că alcaloizii sintetizați de plante au rol de protecție și de apărare ale acestora,

and medicine as therapeutic agents, in agriculture as pesticides as well as other fields.

The following section, **the second**, refers to phenolic compounds which represent a large group of secondary metabolites, including both simple phenols and their derivatives, polyphenols with different functions in growth, development and plants protection. The author mentions various examples of simple phenols such as pigments and flavors that can attract or repel pollinators of flowering plants. It is also granted special attention to secondary metabolites that can protect plants against insects, fungi, bacteria and viruses. Moreover, in this chapter, we find data on the chemical structure of the components the main precursors of lignins, phenyl propane type polymers which form important constituents of the cell walls from plant tissue. This chapter ends with a subchapter discussing various chemical structures of condensed and hydrolysable tannins that because of their ability to bind proteins, they protect plants from being eaten by animals but having other physiological functions in plant life, too. We must not forget the many practical uses of tannins.

Chapter 3 is dedicated glycosides, a chemical compound widely spread in many plant families. Used in both traditional medicine and modern medicine, glycosides exert a wide spectrum of therapeutic actions, ranging from cardiac, analgesic, anti-inflammatory and anti-rheumatic etc.

Chapter 4 comprises informations related to terpenes (terpenoids), natural organic compounds formed from one or more units of isoprene (2-methyl-1,3-butadiene), hence the name of isoprenoids. These substances, known since the ancient times, are spread in plants in the form of active biologically compounds for a wide variety enjoying by diverse practical used as pharmaceuticals, flavors, dyes, perfumes, chemicals used in agriculture etc.

Chapter 5 focuses on essential aspects regarding alkaloids, a large family of secondary metabolites which contain a nitrogen atom in their structure and being found in more than 1200 species of plants. It was also underlined that alkaloids synthesized by plants have a protective role and to defense plant, being toxic to mammals. In small quantities, alkaloids can be used as pharmaceuticals and in

fiind toxici pentru mamifere. În cantități mici, alcaloizii pot fi folosiți ca substanțe farmaceutice, iar în cantități mari ei sunt utilizați ca insecticide și otrăvuri. Mergând mai în profunzime, în acest capitol sunt detaliate răspândirea alcaloizilor, caracteristicile generale ale alcaloizilor, rolul fiziologic al alcaloizilor în plante, clasificarea și biosinteza alcaloizilor, reprezentanții principali ai claselor de alcaloizi în plante sau ciuperci etc.

În capitolul 6 autoarea a inclus date interesante despre uleiurile esențiale, rășini și balsamuri. Astfel sunt indicate organele plantelor care conțin uleiuri esențiale dar și țesuturile specializate pentru producerea și stocarea uleiurilor esențiale la diferite familii de plante. În plus, aici sunt menționați diverși compuși chimici prezenți în uleiurile eterice, rășini și balsamuri.

Cunoscând rolul produselor naturale și a componentelor cu rol de protecție în alimentația omului, de o mare importanță în special în ultimii ani, în **ultimul** capitol autoarea realizează o imagine de ansamblu a metaboliților secundari existenți în legume, fructe, băuturi, condimente și alte plante aromatice utilizate în alimentație. În plus, aici sunt prezentate în detaliu diverse aspecte referitoare la fructe, legume și plante. Fiind considerate cele mai bune „gărzi de corp” pentru sănătatea omului, fructele, legumele și plantele, în general, conțin în plus față de fibre, vitamine și minerale, diferiți compuși chimici bioactivi din categoria metaboliților secundari care au fost denumiți fitochimicale sau fitonutrienți.

În legătură cu aceștia, sunt prezentate date cu privire la dovezile epidemiologice care au arătat corelația pozitivă dintre o dietă bogată în fitonutrienți cu un risc scăzut de cancer, cu o reducere a riscului de boli cardiovasculare, hipertensiune, diabet zaharat și alte maladii. În context, se fac referiri adecvate și la compușii organici existenți în băuturile consumate de om, între care se numără ceaiul, cafeaua, cacao, vinurile, berea și whisky.

Cartea scrisă de doamna lector Lăcrămioară Oprică se distinge printr-un pronunțat caracter interdisciplinar și aplicativ. Prin conținutul său de deosebit nivel științific, prin expunerea clară a problematicii de mare diversitate, această monografie poate fi utilă studenților, masteranzilor și doctoranzilor din domeniile științelor biologice și

large amounts they are used as insecticides and poisons. Going deeper, in this chapter are detailed spread alkaloids, general characteristics of alkaloids, the physiological role of plants alkaloids, classification and their biosynthesis, the main representatives of the alkaloids classes in plants or fungi etc.

In chapter 6 the author included interesting data about *the essential oils, resins and balsams*. Therefore are indicated organ of plants containing essential oils and specialized tissues for the production and storage of essential oils from various plant families. In addition, here are mentioned several chemical compounds presents in essential oil, resins and balsams.

Knowing the role of natural products and constituents as protective of great importance in human nutrition especially in the recent years, in the **final** chapter the author shows an overview of secondary metabolites presents in vegetables, fruits, beverages, spices and other aromatic food plants.

In addition, here are presented in detail various issues related to fruits, vegetables and plants. Being considered the best "bodyguards" on human health, fruits, vegetables and plants generally contain in addition to fiber, vitamins and minerals, various chemicals bioactive compounds from category of secondary metabolites which were named phytochemicals or phytonutrients.

In connection with these, are presented data on epidemiological evidence that showed a positive correlation between a diet rich in phytonutrients with a decreased of cancer risk, with a risk reduction of cardiovascular disease, hypertension, diabetes and other ailments. In this context, there are adequate references to organic compounds present in the human drinks which include tea, coffee, cocoa, wine, beer and whiskey.

The book written by Mrs. lecturer Lăcrămioară Oprică is distinguished by a strong interdisciplinary and applied character. By its special scientific level content, by clear issues of great diversity, this monograph can be useful to students, undergraduate, postgraduate and doctoral students in the fields of biological and medical sciences, as well as researchers and professionals in biochemistry, physiology, genetics, molecular

medicale, dar și cercetătorilor și profesioniștilor din biochimie, fiziologie, genetică, biologie moleculară, farmacie și medicină, agricultură, industria alimentară și industria de substanțe biologice active.

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biology, pharmacy and medicine, agriculture, food industry or pharmaceutical industries.

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