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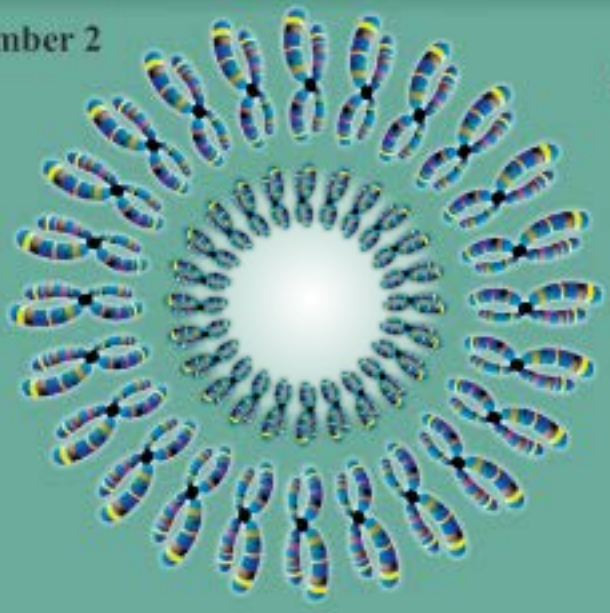

Editura Universității „Alexandru Ioan Cuza" din Iași

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CONTENT

ESTIMATION OF THE EFFECTS OF CHITOSAN-IRON NANOCOMPOSITES DEVELOPED BY DIFFERENT PROCESSES ON *R. GRACILIS* CNMN-Y-30 YEAST

ALINA BEȘLIU[1]

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Keywords: yeasts, chitosan-iron nanocomposites, *Rhodotorula gracilis,* viability, biomass production

Abstract. The paper provides information on the estimation of the effects of iron chitosan nanocomposites, elaborated by different procedures on pigmented yeast *Rhodotorula gracilis* CNMN-Y-30. It was found that the initial amount of chitosan, the concentration of Fe_3O_4 nanoparticles and the volume of nanocomposite used for growing yeasts are the main factors that influence the efficiency of chitosan-iron nanocomposites. Microbiological indices adequately reflect the effects of chitosan-iron nanocomposites in the process of evaluating the action of nanocomposites obtained by different processes on the representative yeast *R. gracilis* CNMN-Y-30 and it is recommended to test the degree of influence of the nanocomposite. This information can be used by specialists in the food industry, microbiology, medicine, cosmetology, environmental protection, etc., where nanocomposites have applications.

INTRODUCTION

Iron oxide nanoparticles (Fe_3O_4) offer attractive possibilities for applications in biotechnology, biomedicine, cosmetology, pharmacology, nutrition, etc. (Blaney, 2007, Shahzeidi et al., 2015). However, high toxicity and low stability produce impediments for use (Puja et al., 2015; Sarlo et al., 2009; Usatîi, et al., 2017). Due to their small size and large surface / volume ratio, the nanoparticles tend to form agglomerations; they also have the ability to easily oxidize in air. It is therefore necessary to modify the surface to stabilize the Fe_3O_4 nanoparticles and to avoid oxidation processes. These shortcomings can be reduced by combining nanoparticles with different polymers, in particular polysaccharides (Silva et al., 2013; Sonaje et al., 2011; Zlotski et al., 2017). Polysaccharides are non-toxic, biodegradable, and biocompatible with the environment. A new approach for broadening the spectrum of use of iron oxide nanoparticles presents their coverage with chitosan. Chitosan is of interest as a biopolymer to efficiently stabilize metal oxide nanoparticles, offering increased biocompatibility and chemical functionality (Dias et al., 2011; Vikele et al., 2017).

The modified surface of nanoparticles in combination with polysaccharides may initiate different effects of the nanocomposite. Generally, chitosan-iron nanocomposites are required in applications in the transport of anticancer drugs (doxorubicin, 5-fluorouracil and leucovorin (Kevin et al., 2001, Tan et al., 2009). They may also participate in the release and administration of cDNA against hepatitis B through the nasal mucosa (Khatri, 2008); protein delivery (Sonaje, 2011), insulin administration (Finotelli et al., 2010), release of polyphenolic antioxidants reduce the cytotoxic effect on living cells (Zheng, 2011) and have antimicrobial effect (Qi, 2004). In biotechnology, the iron chitosan nanocomposite can be applied to immobilization of penicillin G acylase (Xiao-Min Ling, 2016). In remediation of the environment it can participate in the cleaning of the water and soil of persistent organic pollutants (Huang et al., 2015; Tang et al., 2013; Gutierrez et al., 2017).

However, recent advances in investigating the toxicity of nanocomposites have proposed both safety and risk at the same time. However, it was still essential to consider the potential danger posed by nanocomposites with different sizes and concentrations on biological objects (Bui et al., 2017). The influence of iron chitosan nanocomposites can be assessed by means of *R. gracilis* pigmented yeasts, which can serve as a biotechnological object but also as a test model organism. Therefore, the assessment of the more acute and sub-acute toxicity of nanocomposites in vivo should be carried out to avoid potential hazards in the future (Wang et al., 2017).

Taking into account the above, the purpose of the research is to estimate the effects of chitosan-iron nanocomposites, elaborated by different procedures, on yeasts of the genus *Rhodotorula*, in the context of establishing the potential for use.

MATERIALS AND METHODS

Objects of research. Pigmented yeast strain *Rhodotorula gracilis* CNMN-Y-30, producer of proteins and carotenoids, was selected for the research (Usatîi et al., 2016). The strain is preserved in the collection of Yeasts Biotechnology Laboratory and in the Collection of Nonpathogenic Microorganisms of Institute of Microbiology and Biotechnology of Moldova.

Nanomaterials. In the experiments Fe_3O_4 nanoparticles (NPs) 50-100 nm in the form of powder, surface area> 60 m^2 /g, density 4.8-5.1 g/ml at 25^0 C (Aldrich). The nanoparticle stock solution was prepared according to the method outlined by (Otero-Gonzalez et. al., 2013). The NPs suspensions were prepared by the sonochemical method, stabilization was performed in chitosan polymer (Aldrich). Coating of NPs Fe_3O_4 with chitosan was prepared according to the method specified (Mohammadi-Samani et al., 2013).

The experimental procedure I. To 25 mg chitosan was added 25 ml of 1% acetic acid and stirred for 10 minutes at 200 r. p.m. Subsequently, the neutral pH with NaOH is established, 1% of ethyl alcohol of 96% is added. It is stirred for 10 minutes at 200 r.p.m. In the chitosan solution add the Fe_3O_4 nanoparticles (50-100 nm) at 50 mg/L and 70 mg/L and sonify for 10 minutes. During this process chitosan molecules are absorbed on the surface of metal nanoparticles.

The experimental procedure II. To 50 mg chitosan was added 25 ml of 1% acetic acid it is prepared according to the procedure indicated in procedure 1.

Control procedure I. Chitosan-iron nanocomposite is prepared according to the method proposed by Mohammadi-Samani (2013). 20 mg chitosan is dissolved in 1M acetic acid solution with a final volume of 100 ml. Then, 70 mg of Fe_3O_4 nanoparticles is added to the above solution. The mixture is stirred for 18 hours until, a homogeneous dark brown solution is obtained.

Control procedure II. Yeast strain is grown on YPD culture medium, without the introduction of chitosan-iron nanocomposites.

Culture Media. YPD (yeast-peptone-dextrose) fermentation medium and wort was used to obtain the seed material and to grow the yeasts *Rhodotorula gracilis* CNMN-Y-30 (Aguilar-Uscanga et al., 2013).
Submerged cultivation will be carried in Erlenmeyer flasks 1.0 L, the rotating speed of the stirrer 200 rpm, at +25˚…27˚C, the degree of aeration 80.0...83.0 mg/L, permanent lighting 2000 Lx, the time of cultivation 120 hours. Broth medium was seeded in an amount of 5% with the inoculum 2 x 106 cells/mL.
Methods of achieving research. The viability of yeast *Rhodotorula gracilis* CNMN-Y-30 was determined by the microbial counting method which consists in performing the serial dilutions of yeast suspension with subsequent, platingin agarized YPD medium and counting of total colonies. Manual counting was performed (Концевая et al., 2011). Productivity of yeasts biomass was determined gravimetrically (Hong-Zhi et al., 2009).

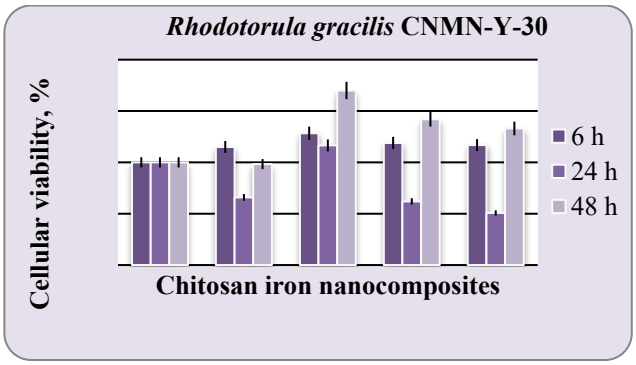
The data results of 3-5 repetitions obtained were expressed by calculating the mean, standard deviation and confidence interval for an average. All differences were considered statistically significant for $P \leq 0.05$, compared to the control variant.

RESULTS AND DISCUSSIONS

The preparation method plays an important role in obtaining nanocomposites adapted for practical applications. Within the experiences presented in this study, was evaluated the action of the chitosan-iron nanocomposite, applied in the cultivation environment in volumes of 2% and 5%, on the cell viability and biomass production in the yeast strain *R. gracilis* CNMN-Y-30.

As a result of the research, it was found that the chitosan-iron nanocomposites prepared according to the experimental procedures I and II, invoke different effects compared to the control procedure proposed by Mohammadi-Samani (2013). The quantification of cell viability in the yeast strain *R. gracilis* CNMN-Y-30 demonstrated the tendency to increase the number of cells only in the variant in which the nanocomposite prepared according to the experimental procedure I in 2% volume was added (fig. 1). Cellular viability increases by up to 16.5% compared to chitosan-iron nanocomposite prepared according to the Mohammadi-Samani control procedure (2013). At the same time, the nanocomposite prepared according to the experimental procedure I, but introduced into the culture medium for yeasts by 5% volume, leads to a statistically reliable reduction (up to 50% compared to the control procedure) of cell viability for

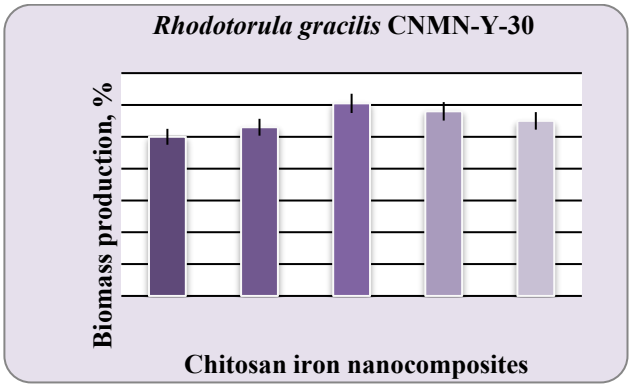
24 hours. Pronounced toxic effect on the viability of yeast cells, showed and nanocomposite prepared according to experimental procedure II used both in volume of 2% and 5%. After 6 and 24 hours of contact with the nanocomposite, the essential reduction of the number of viable cells (by 84-85% compared to the control procedure) is observed (fig. 1).



Legend: 1 - Control (YPD); 2 - Control procedure I; 3,4,5 - experimental procedure I, with the content of Fe_3O_4 nanoparticles in concentration of 30, 50 and 70 mg/L, respectively.

Fig.1. Cellular viability of yeast strain *R. gracilis* CNMN-Y-30 under the action of chitosan-iron nanocomposites, obtained by different processes

The results of the evaluation of the influence of the chitosan-iron nanocomposites obtained by different processes on the production of biomass *R. gracilis* CNMN-Y-30 showed similar changes to those exposed for the viability of the cells. The resulting study shows that in the case of the application of the nanocomposite prepared according to the experimental procedure I, introduced in the culture medium for yeasts by volume of 2%, after 120 hours of cultivation, it initiates the increase of the cellular biomass quantity (fig. 2). The pronounced toxic effect on the accumulation of the yeast biomass exerted the nanocomposite prepared according to the experimental procedure II, which applied in volume of 2% and 5%, causes the essential reduction of the biomass quantity compared to the control process.



Legend: 1 - Control (YPD); 2 - Control procedure I; 3,4,5 - experimental procedure I, with the content of Fe_3O_4 nanoparticles in concentration of 30, 50 and 70 mg/L, respectively.

Fig.2. Biomass production at the yeast strain *R. gracilis* CNMN-Y-30 under the action of chitosan-iron nanocomposites, obtained by different processes.

Thus, the results of the tests showed that the most efficient process for the formation of nanocomposites is the experimental procedure I, which highlighted new aspects regarding the viability and differentiated development of the yeast *R. gracilis* CNMN-Y-30 under the action of the chitosan-iron nanocomposite compared to the cultivation in common conditions. The results of the study contribute to the efficiency of the research on elucidation of the processes or mechanisms of action of the nanocomposite on the metabolism of the yeast cells.

CONCLUSIONS

Generalizing the results obtained in this study it can be mentioned that the the initial quantity of chitosan, the concentration of metallic nanoparticles and the volume of nanocomposite used for the cultivation of yeasts are the main factors that influence the efficiency of the chitosan-iron nanocomposites.

The optimal variant for the preparation of chitosan - iron nanocomposites is the experimental procedure I to 25 mg chitosan was added 25 ml of 1% acetic. For the submerged cultivation of yeasts, the nanocomposite is added to the YPD culture medium in volume of 2%. The microbiological indices adequately reflect the effects of chitosan-iron nanocomposites in the process of evaluating the action of the nanocomposites obtained by different processes on the representative yeast *R. gracilis* CNMN-Y-30 and it is recommended to test the degree of influence of the nanocomposites on the yeasts.

REFERENCES

1. **Aguilar-Uscanga, B., Francois, J.** (2003): *A study of the yeast cell wall composition and structure in response to growth conditions and mode of cultivation*. Letters in Applied Microbiology, 37, 268-274.
2. **Blaney, l.** (2007): *Magnetite (Fe_3O_4): Properties, Synthesis, and Applications.* Lehigh Preserve, 15, 33-81.
3. **Bui, V.K.H., Park, D., Lee, Y. C.** (2017): *Chitosan Combined with ZnO, TiO_2 and Ag Nanoparticles for Antimicrobial Wound Healing Applications: A Mini Review of the Research Trends*. *Polymers, 9,* 21, 1-24.
4. Dias, A. M. G. C., Hussain, A., Marcos, A. S., Roque, A. C. A. (2011): *A biotechnological perspective on the application of iron oxide magnetic colloids modified with polysaccharides.* Biotechnology Advances, 1, 29, 142–155.
5. Finotelli, P.V., Da Silva, D., Sola-Penna, M., Rossi, A.M., Farina, M, andrade L.R., Takeuchi, A.Y., Rocha-Leão, M. H. (2010): *Microcapsules of alginate/chitosan containing magnetic nanoparticles for controlled release of insulin*. Colloids Surf B Biointerfaces. 1, 8, 206-211.
6. Gutierrez, A.M., Dziubla, T.D., Hilt, J.Z. (2017): *Recent advances on iron oxide magnetic nanoparticles as sorbents of organic pollutants in water and wastewater treatment*. Environ Health., 1, 32, 111–117.
7. Hong-Zhi, L., Qiang, W., Yuan-Yuan, L., Fang, F. (2009): *Statistical optimization of culture media and conditions for production of mannan by S. Cerevisiae.* Biotechnology and Bioprocess Engineering, 14, 5, 577-583.
8. Huang,Y.K., Keller, A.A. (2015): *EDTA functionalized magnetic nanoparticle sorbents for cadmium and lead contaminated water treatment. Water Res*., 80, 1, 159–168.
9. Kevin, A., Janes, M.P.F., Marazuela, A., Fabra, A. Alonso, M.J. (2011): *Chitosan nanoparticles as delivery systems for doxorubicin*. Journal of Controlled Release. 73, 255-267.
10. Khatri, K., Goyal, A.K., Gupta, P.N., Mishra, N., Vyas, S.P. (2008): *Plasmid DNA loaded chitosan nanoparticles for nasal mucosal immunization against hepatitis. International Journal of Pharmacy,* 1-2, 354, 235-41.

11. Mohammadi-Samani, S., Miri, R., Salmanpour, M., Khalighian, N., Sotoudeh, S., Erfani, N. (2013): *Preparation and assessment of chitosan-coated superparamagnetic Fe_3O_4 nanoparticles for controlled delivery of methotrexate*. Res Pharm Sci, 1, 8, 25–33.
12. Otero-Gonzalez, L., Garcia-Saucedo, C., Field, G., Sierra-Alvarez, R. (2013): *Toxicity of TiO2, ZrO2, Fe0, Fe2O3 and Mn2O3 nanoparticles to the yeast, Saccharomyces cerevisiae.* Chemosphere, 93, 1201-1206.
13. Puja K., Cynthia O., Boon H., Gyeong H. (2015): *Nanotoxicity: An Interplay of Oxidative Stress,* Inflammation and Cell Death. Nanomaterials, 5, 1163-1180.
14. Qi, L., Xu, Z., Jiang, X., Hu, C., Zou, X. (2004): *Preparation and antibacterial activity of chitosan nanoparticles.* Carbohydrate Research., 16, 339, 2693-700.
15. Sarlo, K., Blackburn, K.L., Clark, E.D., Grothaus, J., Chaney, J., Neu, S., Flood, J., Abbott, D., Bohne, C., Casey, K. (2009): *Tissue distribution of 20 nm, 100 nm and 1000 nm fluorescent polystyrene latex nanospheres following acute systemic or acute and repeat airway exposure in the rat.* Toxicology, 263, 117–26.
16. ***Shahzeidi, I Z.S., Amiri, G. (2015): Antibacterial activity of Fe_3O_4 nanoparticles.*** *In: Bio-Inorg.* Hybrid Nanomaterials, 4, 3, 135-140.
17. **Silva, V., Andrade, M. P. C., Silva, A. Bustamante, D., Valladares, L. L. S., Aguiar, A.J.** (2013): *Synthesis and characterization of Fe_3O_4 nanoparticles coated with fucan polysaccharides.* In: *Journal of* Magnetism and Magnetic Materials, 343, 138–143.
18. Sonaje, K., Kun, J. L., Tseng, M.T., Shiaw, P.W., Fang, Y., Yuan, C., Chia, W. (2011): *Effects of chitosan-nanoparticle-mediated tight junction opening on the oral absorption of endotoxins*. Biomaterials, 33, 32, 8712-21.
19. Tan, M.L., Choong, P.F., Dass, C.R. (2009): *Review: doxorubicin delivery systems based on chitosan for cancer therapy. Pharm Pharmaco*logy, 2, 61, 131-42.
20. Tang, S.C., Lo, I.M. (2013): *Magnetic nanoparticles: Essential factors for sustaianble environmental applications*. *Water Research*, 8, 47, 2613–2632.
21. **Usatîi A., Beşliu A., Chiriţa E**. (2016): Caractere fenotipice şi compoziţia biochimică a tulpinii de levuri pigmentate Rhodotorula gracilis CNMN-Y-30. Conferinţa Tehnico-Ştiinţifică a Colaboratorilor, Doctoranzilor şi Studenţilor, 26 noiembrie 2015 a Univ. Tehn. A Moldovei, 2, 31-35.
22. Usatîi, A., Chiselița, N., Bejenaru, L., Beșliu, A., Efremova, N., Tofan, E. (2017): *The action of TiO_2, ZnO, Fe_3O_4 nanoparticles on Saccharomyces and Rhodotorula yeast strains in function of the concentration and dimensions.* Analele Științifice ale Universității „Alexandru Ioan Cuza*",* Secțiunea Genetică și Biologie Moleculară*,* 18, 2, 65-
23. Vikele, L., Laka, M., Sable, I., Rozenberga, L., Grinfelds, U., Zoldners, J., Passas, R., Mauret, E. (2017): *Effect of chitosan on properties of paper for packaging*. *Cellulose Chemistry* and *Technology.* 1-2, 51, 67-73.
24. Wang, Y., Y$, C.X., Yan, X.P. (2017): *Hydrothermal and biomineralization synthesis of a dual-modal nanoprobe for targeted near-infrared persistent luminescence and magnetic resonance imaging. Nanoscale,* 9, 9049–9055.
25. Xiao-Min, L., Xiang-Yu, W., Ping, M. Y. Y., Jie-Mei, Q., Xue-Jun, Z., Ye-Wang, Z. (2016): *Covalent Immobilization of Penicillin G Acylase onto $_{Fe3O4}$@Chitosan Magnetic Nanoparticles.* Microbial Biotechnology, 5, 26, 829-836.
26. Zheng, A., Hui-Xue, L., Lan, Y., Meng, M. (2011): *Comprehensive studies on the interactions between chitosan nanoparticles and some live cells*. Journal of Nanoparticle Research, 10, 13, 4765-4776.
27. Zlotski, S.V., Uglov, V.V. (2017): *Facile Sol-gel Synthesis of Metaloxide Nanoparticles in a Cellulose Paper Template*. Nanomed Nanotechnology, 8, 1-3.
28. Концевая, И.И. Микробиология. (2011): *Практическое пособие для студентов специальности Биология (научнопедагогическая деятельность).* Гомель: УО ГГУ им. Ф. Скорины, 126.

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A SENSITIVE METHOD FOR SALIVA DETECTION IN FORENSICS USING SALIVARY AMYLASE COUPLED WITH AMPLEX RED OXIDATION

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Keywords: Forensic Science, salivary amylase, Amplex Red, saliva detection, fluorimetry, body fluids detection, serology
Abstract: A new sensitive method for saliva detection was developed, based on salivary amylase detection but with a final fluorescent product, which increases its sensitivity. After the starch is degraded due to the presence of salivary amylase, glucose is oxidised and generates hydrogen peroxide which is able to transform Amplex Red in resorufin - a highly fluorescent product. The final product is visible both under normal and UV light. The method is fast, accurate, can detect trace amounts of saliva and shows little to no interference with other body fluids. A further increase in sensitivity could be obtained by using horseradish peroxidase in the final step, but this would also lead to an increased background signal and stronger interference with urine.

INTRODUCTION

Among the body fluids analysed in forensic science, saliva is one of the most frequently encountered, being a good source of DNA for subsequent typing (Kuwayama et al., 2016; Carboni et al., 2014; Aps and Martens, 2005). In various casework analysis, saliva traces can be found on cigarette butts, clothing, bite marks as well as different objects found at crime scenes. Saliva identification and subsequent genetic analysis remain crucial evidence in court (Groschl, 2017; Saxena and Kumar, 2017).

There are several choices for saliva detection, each of them using a specific marker. Among the various markers proposed in literature (Nakanishi et al., 2009; Virkler and Lednev, 2009), salivary amylase is the most frequently used. There are some advantages, such as: good sensitivity, relative specificity and cost effective detection. There are also a few drawbacks, including the possibility of obtaining false positive results and the inability to differentiate between species (Saxena and Kumar, 2017).

Although the amylase function was described as early as 1831 by E.F. Leuchs (Zakowski and Bruns, 1985) it wasn't used in casework until 1928 (Mueller, 1928). The enzyme is found in various body fluids (saliva, blood, urine, semen etc.), but the highest concentration is encountered in saliva (10). The biochemical function of this enzyme is to hydrolyse the α-(1,4)-glucoside bonds found in a variety of polysaccharides and this breakdown reaction could be the starting point in saliva identification.

Among the amylase based methods used for saliva identification, many rely on colour changes (the radial diffusion test (Quarino et al., 1993) , Phadebas® test (Wornes et al., 2018) and SALIGaE® test (Park et al., 2015)) which are based on substrate chemical changes after amylase action. The oldest variant uses starch/ iodine for detection of amylase (Myers and Adkins, 2008) and the most common tests today use a dextrin linked to a 4-nitro-phenol moiety (4-nitrophenyl-maltoheptaoside - which releases 4-nitro-phenol, with a yellow colour) (Soyama and Ono, 1983) or a substrate made of insoluble starch coloured blue with a dye marker (Ceska et al., 1969). Some other methods are based on antibody-antigen interactions and have the advantage of human specificity (RSID) (Old et al., 2009).

The present study aims to increase the sensitivity of the amylase based saliva identification method, using enzymatic reactions that are connected to a final highly fluorescent product (Figure 1). The salivary amylase is able to hydrolyse a starch solution with subsequent release of glucose and dextrin formation. The amount of glucose could be increased by adding α-glucosidase. In the next step, glucose-oxidase transforms glucose in D-gluconolactone and hydrogen peroxide. The final step consists of detection of hydrogen peroxide: Amplex Red will be transformed into a highly fluorescent compound – resorufin (oxidation, de-acetylation and double bond rearrangement), the transformation being assisted by horseradish peroxidase. It is worth mentioning that resorufin is also visible under normal light, as a bright pink compound.

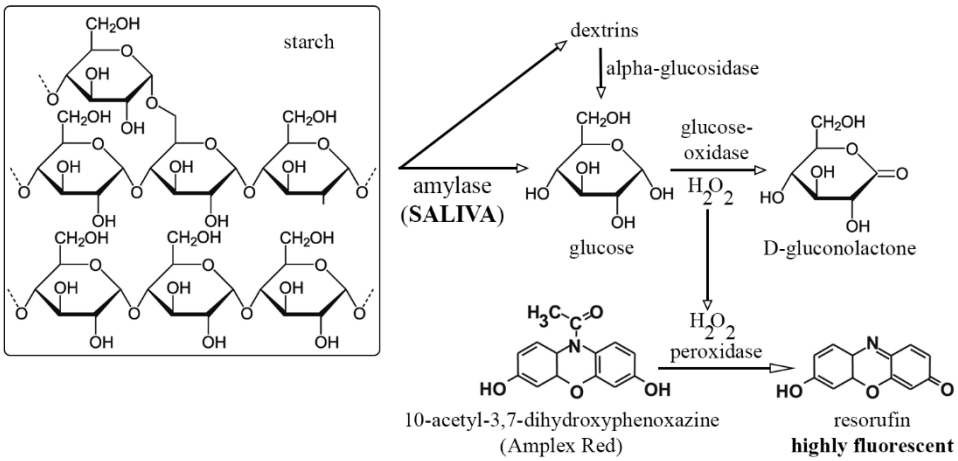

Figure 1. The proposed principle of saliva detection.

MATERIALS AND METHODS

Chemicals. Amylase, glucose oxidase, α-glucosidase, horseradish peroxidase, DMSO and Amplex Red were purchased from Sigma Chemical Co. (St. Louis, MO, USA). After testing three different types of starch, an in-house made soluble form of starch was used (Han and Lim, 2004): 50 g of food grade corn starch were dissolved in 100 ml dimethyl sulfoxide (DMSO) and kept under mild stirring at 37^0 C for 24 hours. Using this procedure, a high percent of the amylose chains is unfolded, resulting in increased solubility and greater susceptibility for amylase. This DMSO modified starch was precipitated with 200 ml cold ethanol 99.8%, vacuum filtered and washed three times with cold ethanol 99.8% and then used as a substrate for the saliva detection experiments. After drying, the modified starch was stored at room temperature and dissolved before conducting the experiments. A glucose-oxidase based kit for glucose detection from Biosystems (Barcelona, Spain) was used for checking the starch quality.

The proposed protocol for saliva detection. In a test tube, the following solutions were mixed: 100 µL saturated solution of the described above soluble starch, 100 µL fresh saliva obtained from a healthy volunteer, 50 µL α-glucosidase (0,2 mg of solid enzyme with 23 units/mg in 1000 µL water) and 25 µL glucose-oxidase (0,8 mg of solid enzyme with 175 units/mg in 1800 µL water). The mixture was vortexed for 20 seconds and incubated at room temperature for 4 minutes. The final step consisted in adding 10 µL of Amplex Red solution (0,8 mg dissolved in 1000 µL dimethyl sulfoxide – DMSO) and 4 µL horseradish peroxidase solution (1,6 mg of solid enzyme with 113 units/mg in 1800 µL water). The final mixture was vortexed for 20 seconds and incubated at room temperature for another 4 minutes.

Spectrophotometric measurements. The resorufin concentration obtained in the presence of saliva was measured using a Piccos Biochemistry Analyser (AMP Diagnostics, Belgium) with a 546 nm filter, taking into account that resorufin light absorbance is near 550 nm (Silva et al., 2016).

Fluorimetric measurements. All the measurements were made using an EnSight Multimode Plate Reader (Perkin Elmer, USA) with a 560 nm excitation wavelength and a 588 nm emission wavelength. Using a 96 well plate, serial dilutions of saliva were analysed in triplicate at different time frames.

Interference with other biological fluids. Different saliva samples were mixed in variable proportions with urine, blood or diluted blood, and serum (mixtures saliva/other biological fluids were 1/3, 1/1 and 3/1 for each fluid). The mixtures or the body fluids alone were then analysed with the proposed method.

RESULTS AND DISCUSSIONS

Influence of starch type used as a substrate for salivary amylase. After testing three different types of starch (Sigma soluble starch, alimentary grade starch and in-house made soluble starch), the best results were obtained with a dissolved and re-precipitated form of corn starch, as described

in materials. Using a glucose-oxidase based kit for glucose detection this type of starch was the only one that was still clearly detectable at 0.02 mg/mL concentration.

Time steps optimisation. Several incubation periods were tested in order to obtain the best colour and fluorescence signal. After changing the time intervals for step 1 from 1 to 10 minutes and from 1 to 20 minutes for step 2, the best incubation times were selected: 4 minutes for step 1 and 4 minutes for step 2. The colour and fluorescence intensity increased after 8 minutes (Figure 2) but this also led to an increase in the negative control. The results were still visible after 24 hours, but with an even stronger increase in intensity for the negative control.

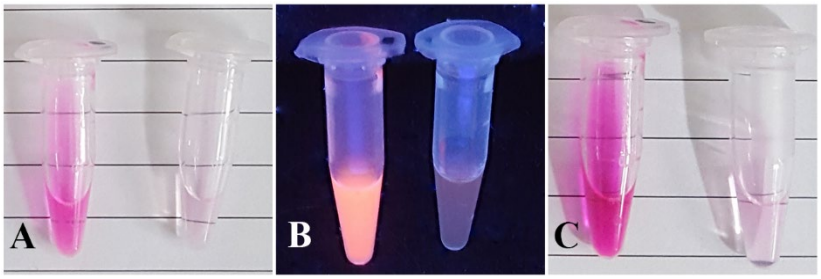

Figure 2. Colour and fluorescence changes with/ without 100 µL saliva – after 4 minutes in visible light (A); after 4 minutes in UV light, 365 nm (B); after 8 minutes in visible light (C).

Limits of detection. The minimum amount of saliva detectable with this method was measured using three different methods: spectrophotometry, fluorimetry and visual macroscopic examination.

Using PBS buffer solution for serial dilutions of saliva ranging from 100 to 0.19 µL/test, the spectrophotometric measurement was able to detect as little as 0,78 µL (Figure 3a). Interestingly, almost the same volume of saliva can also be detected by direct visual examination (Figure 4).

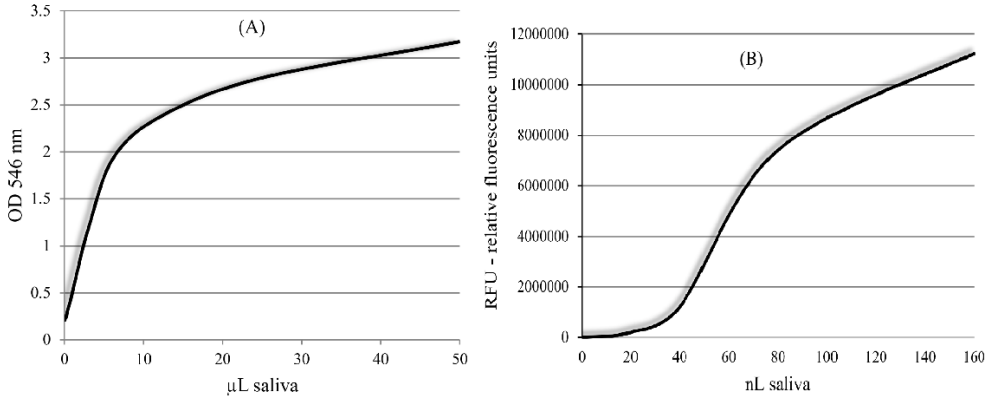

Figure 3. Measurements of light absorbance (OD – optical density) at 546 nm **(A)** and fluorescence intensity - 560 nm excitation and a 588 nm emission - **(B)** for serial dilutions of

saliva. Values are the means of three determinations, and the standard deviation was below 7 % of the mean.

For fluorimetric measurements (Figure 3b) the lowest detection limit reached 20 nL, this result being undetectable using direct examination under UV light. With a saliva volume of 20 nL, the fluorescence signal was still 10 times higher than the negative control.

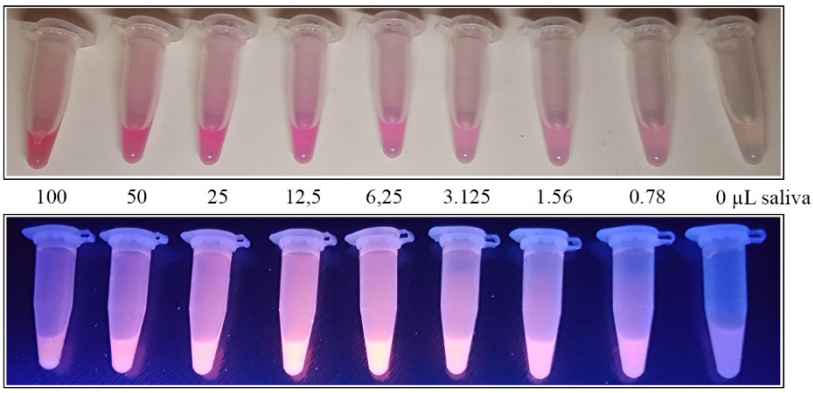

Figure 4. Serial dilution of saliva (ranging from 100 to 0,78 µL) – direct examination under visible and UV light (365 nm).

Interference with other body fluids. As expected, the body fluids that contain certain amount of amylase were able to produce interference with the proposed method of saliva detection. Indeed, after testing various body fluids (alone or mixed with saliva) it was established that the interference was negligible for blood, diluted blood or serum and was stronger for urine. The urine interference could be an important drawback for the method, since the history of forensic cases depicts real situations when these two body fluids must be differentiated. However, this problem could be solved if the final horseradish peroxidase is completely removed from the protocol and the second step incubation time is increased from 4 minutes to 6 minutes. Despite a minor decrease in sensitivity (data not shown), the method without peroxidase was able to make a visible distinction between saliva and urine. Since the removal of peroxidase was the solution for interference, it seems that the problem came from some unwanted substrates found in urine (which are able to produce hydrogen peroxide in the presence of peroxidase) and not necessarily from the urinary amylase. Also, the proposed method was still usable in cases of old saliva (1-28 days) with a minimum decrease of sensitivity, facts which are consistent with literature data (Tsutsumi et al., 1991).

CONCLUSIONS

The method described above could be an interesting alternative for saliva identification in forensic science. The method is sensitive, fast and with little to no interference with other body fluids. Despite being based on a “classical” marker – salivary amylase -, this new method brings at least new standards of sensitivity due to the use of a highly fluorescent final compound visible both in

normal and UV light conditions. This feature could be useful to detect saliva traces on dark surfaces, due to the highly fluorescent properties of the final compound.

REFFERENCES

Aps, J. K., Martens, L. C. (2005): *Review: The physiology of saliva and transfer of drugs into saliva.* Forensic Sci Int, 150(2-3):119-31.
Carboni, I., Rapi, S., Ricci, U. (2014): *Stability of human alpha-salivary amylase in aged forensic samples*. Leg Med (Tokyo), 16:214-17.
Ceska, M., Birath, K., Brown, B. (1969): *A new and rapid method for the clinical determination of α-amylase activities in human serum and urine. Optimal conditions.* Clin Chim Acta, 26(3):437-44.
Groschl, M. (2017): *Saliva: a reliable sample matrix in bioanalytics*. Bioanalysis, 9(8):655-68.
Han, J. A., Lim, S. T. (2004): *Structural changes of corn starches by heating and stirring in DMSO measured by SEC-MALLS-RI system*. Carbohydr Polym, 55:265-72.
Kuwayama, K., Miyaguchi, H., Yamamuro, T., Tsujikawa, K., et al. (2016): *Effectiveness of saliva and fingerprints as alternative specimens to urine and blood in forensic drug testing.* Drug Test Anal, 8(7):644-51.
Mueller, B. (1928): *Über den Nachweis eingetrockneten Speichels in Tüchern*. Dtsh Z Gerichtl Med, 11:211-24.
Myers, J. R., Adkins, W. K. (2008): *Comparison of modern techniques for saliva screening*. J Forensic Sci, 53(4):862-7.
Nakanishi, H., Kido, A., Ohmori, T., Takada, A., et al. (2009): *A novel method for the identification of saliva by detecting oral streptococci using PCR.* Forensic Sci Int, 183(1-3):20-3.
Old, J. B., Schweers, B. A., Boonlayangoor, P. W., Reich, K. A. (2009): *Developmental validation of RSID-saliva: a lateral flow immunochromatographic strip test for the forensic detection of saliva.* J Forensic Sci, 54(4):866-73.
Park, H. Y., Son, B.N., Seo, Y. I., Lim, S. K. (2015): *Comparison of Four Saliva Detection Methods to Identify Expectorated Blood Spatter*. J Forensic Sci, 60(6):1571-6.
Quarino, L., Hess, J., Shenouda, M., Ristenbatt, R. R., et al. (1993): *Differentiation of alpha-amylase from various sources: an approach using selective inhibitors.* J Forensic Sci Soc, 33(2):87-94.
Saxena, S., Kumar, S. (2017): *Saliva in forensic odontology: A comprehensive update*. J Oral Maxillofac Pathol: JOMFP, 19(2):263-65.
Silva, F. S., Starostina, I. G., Ivanova, V. V., Rizvanov A. A., et al. (2016): *Determination of Metabolic Viability and Cell Mass Using a Tandem Resazurin/Sulforhodamine B Assay*. Curr Protoc Toxicol, 68(4):2.24.1-15.
Soyama, K., Ono, E. (1983): *Pancreatic and salivary amylase determination using a short-chain chromogenic substrate (alpha-4-nitrophenyl-maltoheptaoside) and an amylase inhibitor.* Clin Chim Acta, 131(1-2):149-54.
Tsutsumi, H., Higashide, K., Mizuno, Y., Tamaki, K., Katsumata, Y. (1991): *Identification of saliva stains by determination of the specific activity of amylase.* Forensic Sci Int, 50(1):37-42.
Virkler, K., Lednev, I. K. (2009): *Analysis of body fluids for forensic purposes: from laboratory testing to non-destructive rapid confirmatory identification at a crime scene*. Forensic Sci Int, 188(1-3):1-17.
Wornes, D. J., Speers, S. J., Murakami, J.A. (2018): *The evaluation and validation of Phadebas® paper as a presumptive screening tool for saliva on forensic exhibits*. Forensic Sci Int, 288: 81-8.
Zakowski, J. J., Bruns, D. E. (1985): *Biochemistry of human alpha amylase isoenzymes*. Crit Rev Clin Lab Sci, 21(4):283-322.

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MICRONUCLEUS TEST IN EPITHELIAL CELLS FROM ORAL CAVITY IN KOYA UNIVERSITY STUDENT SMOKERS AND NON-SMOKERS

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Keywords : epithelial cells , Cigarettes , micronucleated cell, binucleated cell and condensed chromatin cell
Abstract : This work aimed to investigate the use of epithelial cells from the oral cavity in identifying smoking-related effects in male smokers, normal male, normal female. To establish the relationships between micronucleated cell, binucleated cell, condensed chromatin cell. A total of 59 subjects, corresponding to 11 normal males, 19 normal female, 29 male smokers were registered for this study. The buccal epithelial cell was selected because of the direct exposure of tobacco smoke.
We appraised the incidence of micronucleus formation from 29 male smokers and who had smoked a minimum of 1 pack-year and a maximum of 12. Because of their increased smoke intake, male smokers group showed high buccal micronuclei frequency, significantly $P<0.05$ increased micronucleus frequency was observed in the male smokers group. Micronuclei are cytoplasmic chromatin mass with the appearance of small nuclei that arise from chromosome fragments in the anaphase stage of cell division. Their presence in cells is a reaction of structural and numerical chromosomal aberration arising during mitosis.
In an analysis of the frequency of Binucleated cell in 29 male smokers, 11 normal male statistically non-significant differences were noted. The average frequency of condensed chromatin cell in 11 normal male and 29 male smokers were high $P<0.05$, this is statistically significant and there is a relationship between smoking and increasing in condensed chromatin cell as we mentioned before smoking leads to cytogenetical damage to the human buccal epithelial cell.

INTRODUCTION

Exposure to genotoxic agents occurs through a variety of situations, including pollution of the natural environment, medical procedures (chemotherapy, radiotherapy, etc.) as well as life style factors such as work, diet (Błaszczyk *et al.,* 2014). tobacco smoking, Cigarette smoking is responsible for a substantial number of human health problems (Christobher et al .,2016). One of the major constituents of environmental toxins is tobacco smoke which is responsible for deaths throughout the world . The process of aberrant mitosis gives rise to micronucleus (Ahmad *et al.,* 2015).The oral epithelial cells represent a target site for earlier genotoxic events induced by carcinogenic agents entering the body via inhalation and ingestion. Buccal mucosa cells are the first barrier which are capable of metabolizing carcinogens to reactive products (Yee *et al.,*2015) .
MNi originate from chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division (Fenech 2007). Micronuclei originate from chromatin which for different reasons has been lagging in anaphase . In the course of telophase this material is included into one or the other daughter cell where it either can fuse with the main nucleus or form one or several secondary nuclei (Schmid 1976).The micronucleus assays have emerged as one of the preferred methods for assessing chromosome damage because they enable both chromosome loss and chromosome breakage to be measured reliably(Fenech 2000). Hence micronuclei assay can be used as one of the biomarkers of oral cancer, as it is increased in oral neoplastic conditions. Micronucleus can be identified by various special stains in exfoliative cytology (Suganya *et al .,*2019).
Micronuclei can be identified depending upon following criteria: Cell containing one or more nuclear like substance along with the main nucleus , Each Micronuclei will have the diameter less than 1/3rd of the nucleus , Micronuclei will have oval or circular shape along with membrane , Micronuclei will be located within 3 or 4 nuclear diameters arounda nucleus and will not be in contact with the nucleus and Micronuclei will exhibit similar focal plane, texture and even almostsimilar staining intensity as that of the main nucleus (Vipul *et al.,*2017 and Raj *at al.,* 2019).

MATERIAL AND METHODS

2:1 SUBJECTS :
Subjects (n=59) were the koya university from Kurdistan region of iraq . The foremost inclusion criteria in the present study embrace the analysis Age in year, Body mass index ,Cigarettes per day and Years of smoking. All the controls were physically and mentally normal subjects who had no history of any genetic disorders.

2:2 SAMPLE COLLECTION :
Buccal cell were collected from Smokers and Non-Smokers, Buccal cells were collected from both sides of cheeks by using sterile wooden swab.One swab was used for each cheek and collected the epithelial cell by rotating the wooden swab.

2:3 PROCEDURE :
1-Ask the students to wash their mouth with sterile water
2-Collect the buccal cell by gentle scrapping of wooden swab on their cheek.
3-Spread the swab that contain the collected sample on a clean slideStain the slide by 1% methyl blue then allow the slide to dry for(15 – 20) minute.
4-After drying the slide examine it under the microscope at 40 or 100 X
5-Count 100 cell on the slide under the microscope and detect the presence of micronuclus , Binuclus and condensed nucleus out of 100 cells

2:4 STATISTICAL ANALYSIS :
The statistical significance of the differences in the frequencies-genotypes between groups was calculated. Mean, Standard error of the mean and p-value were calculated to assess the difference between the male smokers and non-smokers and also between normal male and female the level of significance was calculated by t test calculator.

RESULTS :

Table 3: 1 Distribution of groups based on normal males and females with male smokers

Groups	Number	Percentage
Normal male	11	18.64%
Normal female	19	32.20%
Male smoker	29	49.15%
Total	59	100%
Groups	**Number**	**Percentage**
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Normal female	19	32.20%
Male smoker	29	49.15%
Total	59	100%

Table 3:2 General Characteristics of the smoker group and nonsmoker groups

Variable	Male smoker	Mean ± SEM	Normal male	Mean ± SEM	Normal female	Mean ± SEM
Age in year	18-25	21.0± 0.30	18-24	21± 0.77	17-21	19.89±0.27
Body mass index	15.9-32.3	21.21±0.66	20.7-26.7	24.02± 1.03	12-34.6	21.37± 1.01
Cigarettes per day	10-90	24.10±1.82				
Years of smoking	1-12	5±0.54				

Table 3 :3Mean frequency of micronuclei , binucleated cells , Condensed chromatin cells and SEM in buccal epithelium cells of male smokers and normal males

Types of cell	Mean ± SEM of male smoker	Mean ± SEM of normal male	p-value

Micronucleated cells	2.82±0.26	0.45±0.28	0.0001
Binucleated cells	1.89±0.19	1.3±0.20	0.0849
Condensed chromatin cells	0.86±0.19	0.18±0.12	0.0397

Table 4:4 Mean frequency of micronuclei, binucleated cells, Condensed chromatin cells and SEM in buccal epithelium cells of normal males and females

Types of cell	Mean ± SEM of normal male	Mean ± SEM of normal female	p-value
Micronucleated cells	0.45±0.28	0.34±0.14	0.6977
Binucleated cells	1.3±0.20	0.52±0.15	0.0040
Condensed chromatin cells	0.18±0.12	0.11±0.07	0.6327

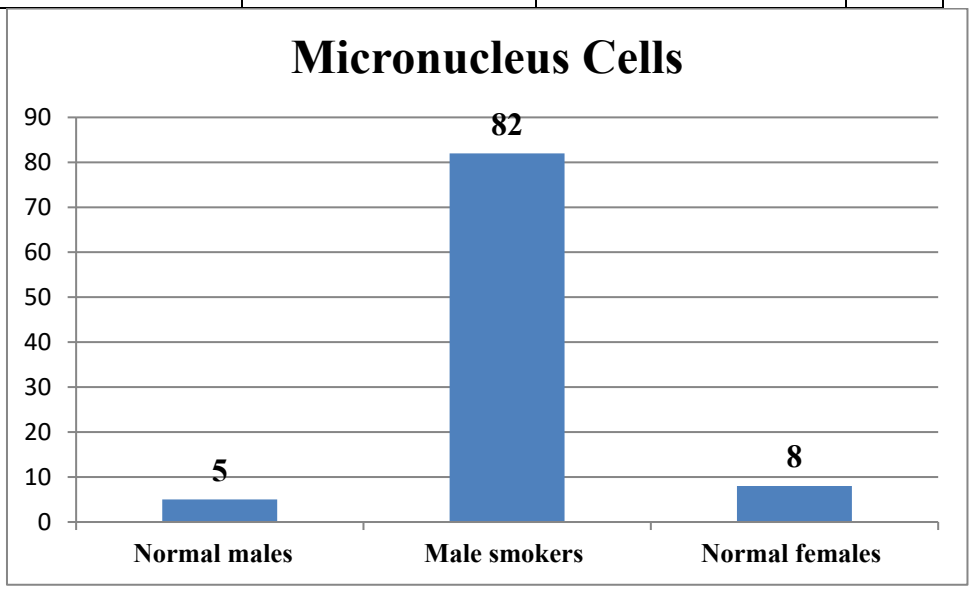

Figure 1:1 total number of micronucleus cells among different groups of students

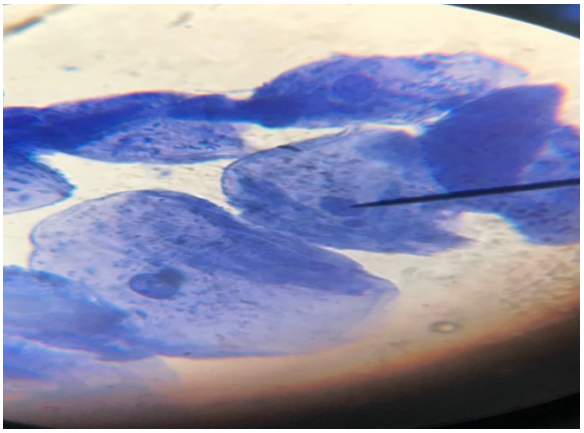

Figure 2:2 micronucleus cells at 100 X of light microscope

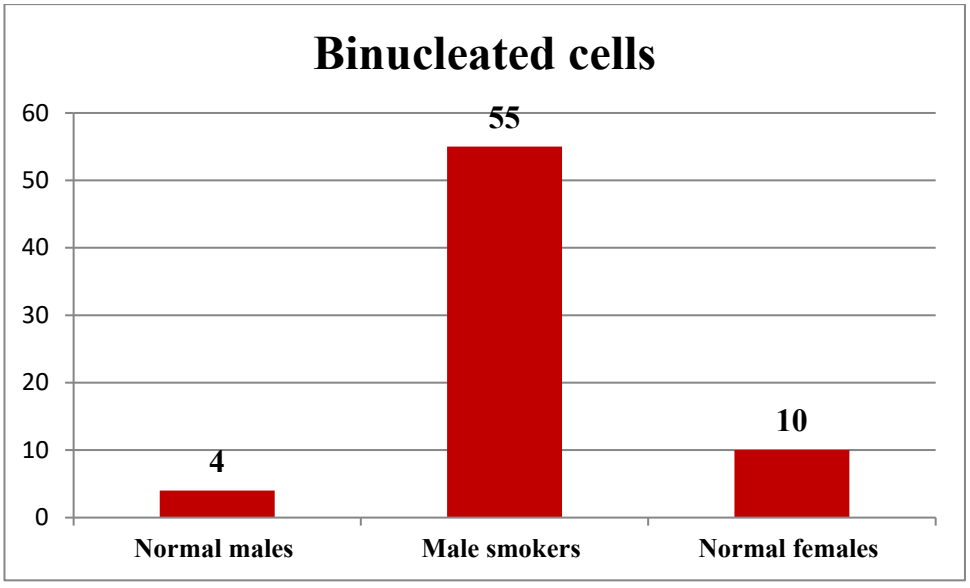

Figure 3:3 total number of binucleated cells among different groups of students

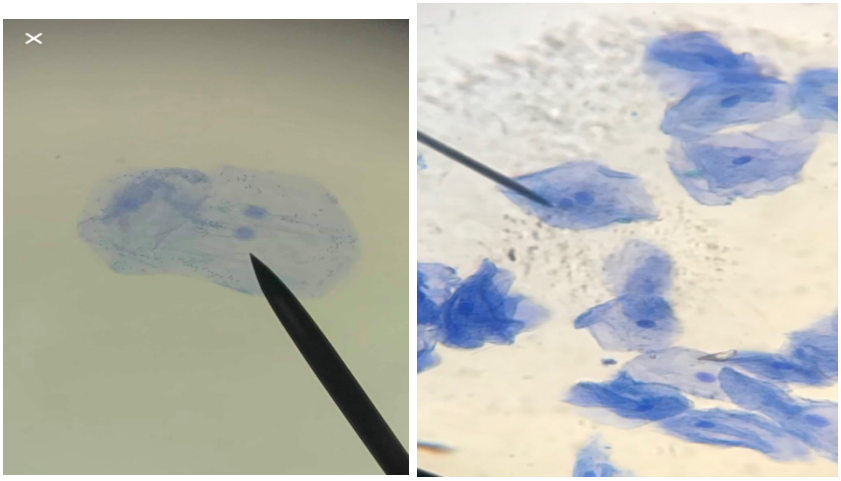

Figure 3:4 binucleated cells at 100 X of light microscope

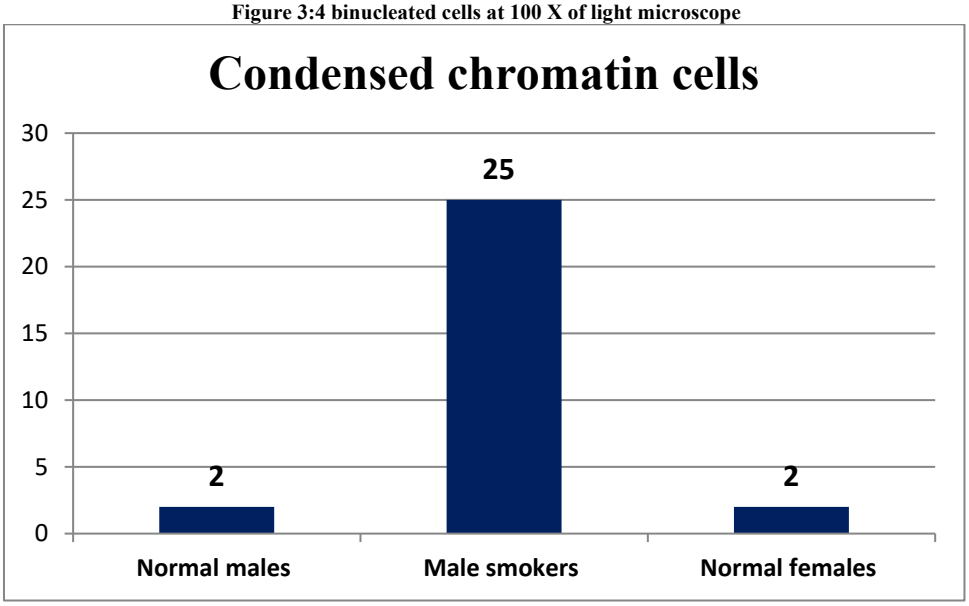

Figure 3:5 total number of Condensed chromatin cells among different groups of students

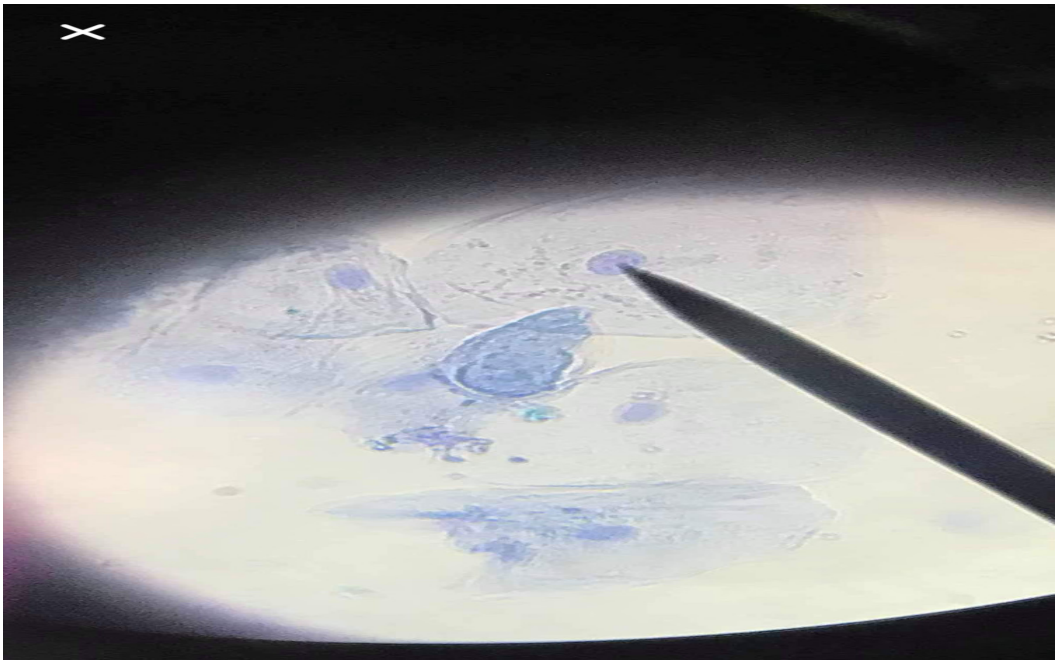

Figure 3:6 Condensed chromatin at 100 X of light microscope

DISCUSSION :

Our study aimed to create relationships in male smokers, normal males and normal females among micronucleated cells, binucleated cells, condensed chromatin cells. A total of 59 participants were registered for this study, corresponding to 11 normal males with 18.64%, and 19 normal females with 32.20% and 29 male smokers with 49.15% (Table 1). Our research aimed to develop the relationships in male smokers, normal males and normal females between micronucleated cells, binucleated cells, condensed chromatin cells. A total of 59 subjects, corresponding to 11 normal males, 18.64%, and 19 normal females, 32.20%, and 29 male smokers, 49.15% (Table 1).

In (Table 2) we showed general characteristics of smoker-group and non-smoker groups. We estimated many categories for evaluating our work such as Age in the year, Body mass index, Cigarettes per day, years of smoking. The age of male smokers is between(18-25) years, the mean age and standard error of the mean of male smokers are (21.0 ± 0.30). Body mass index of male smokers between (15.9±32.3) the mean body mass index and SEM of male smokers are(21.21 ± 0.66). The range of Cigarettes per day in the male smoker is (10 – 90) Mean and SEM is(24.10 ±1.82). years of smoking in male smokers is(1-12) year, mean and SEM is(5+± 0.54). Age of normal male is between(18-24) years the mean and SEM of normal male age is(21± 0.77 Body mass index of a normal male between(20.6-26.7). the mean and SEM of is (24.02 ± 1.03). The age of the normal female is between(17-21) years, the mean and SEM of normal female age is (19.89±0.27). Body mass index of a normal female between(12- 34.6), the mean and SEM of Body mass index of the normal female is (21.37 ± 1.01).

In our study, the average frequency means and SEM of micronuclei in male smoker buccal cells was 2.82±0.26 but the average frequency mean and SEM of micronuclei in normal male is 0.45±0.28 (Table 3). If the P-value equal or less than 0.05 it is statistically significant. The P-values between the male smoker and normal male results is 0.0001 this is significant and there is a relationship between smoking and increasing micronucleated cell in buccal epithelial cell. Bonassi *et al.* (2011). Micronucleus increased with increasing smoking, Błaszczyk et al., 2014 In cells, the molecular and chromosomal changes lead to the formation of micronuclei according to Fenech *et al.* (2011) male smokers buccal epithelial cell showed a higher frequency of micronuclei than normal males due to the increased pack-years and smoke consumption rate. Figure 1 showed the total number of micronucleus cell among different groups of students. Micronucleus in 29 male smokers highly increased, its 82 in number while micronucleus in 11 normal males is 5 in number also 8 in number in 19 normal females. The result is very near between normal males and females. The average frequency mean and SEM of Binucleated cell in male smokers buccal cell are 1.89±0.19 but the average frequency mean and SEM of Binucleated cell in normal males is 1.3±0.20 (Table 3) The P-value between male smokers and normal male results is 0.0849 which is not important there is no relation between smoking and that Binucleated cell. The total number of binucleated cells in 29 male smokers shown in Figure 2 binucleus is 55 in size, and it is 4 in 11 normal males, and is 10 in 19 normal females. Diler and Celi also documented the lack of statistically significant differences in the binucleus frequency in oral cells of male smokers and the average male and the normal male was also reported by Diler and Celik 2011).

Mean and SEM of condensed chromatin cell in male smoker was 0.86 ±0.19, but in normal males was 0.18± 0.12 the P-value between male smoker and normal male results is 0.0397(table 3) which is significant and there is the relationship between smoking and increasing condensed chromatin cell. Figure 3 shows us the total number of condensed chromatin cells and its 25 in number in 29 male smokers, 2 in 11 normal males and 2 in normal females. Condensed chromatin cell results from same between normal male and normal female but its more in male smokers, that show us the effect of smoking on a buccal cell which caused having more condensed chromatin cell.

In Table 4 we explained mean frequency and SEM in the buccal cell of normal males and females. mean and SEM of the micronucleated cell of the normal male is 0.45 ±0.28 and in a normal female is 0.34 ± 0.14. P-value of a micronucleated cell between normal male and normal female is 0.6977 which is not significant, mean and SEM of the binucleated cell of the normal male is 1.3± 0.2 and normal female is 0.52± 0.15. P-Value of the binucleated cell between normal male and normal female is 0.0040 which is significant and there is a relationship between normal male and normal females in binucleated cell number. Mean and SEM of condensed chromatin cells is 0.18± 0.12 in normal male and 0.11± 0.07 in the normal female the P-Value between normal male and normal female for condensed chromatin is 0.6327 which it is not significant.

CONCLUSION

We concluded, as a final conclusion from the result:

1-The relationship between smoking and growing micronucleated cells is important.

2- The lack of statistically relevant variations in the frequency of binucleated cells in male and regular male oral cells;

3- The relationship between smoking and an increase in condensed chromatin cells is significant.

REFERENCES

Ahmad, K.K., Mustafa, S.K. and Karim, J.K., 2015. Prevalence of micronucleated cell in buccal smears among smokers and non-smokers. *International Journal of Advanced Research*, *3*(4), pp.972-977.
Błaszczyk, E. and Mielzynska-Svach, D., 2014. Micronucleus assay in epithelial cells from the oral cavity and urinary tract in female smokers and non-smokers. *Environmental Biotechnology*, *10*(2).pp:60-65
Błaszczyk, E. and Mielzynska-Svach, D., 2014. Micronucleus assay in epithelial cells from the oral cavity and urinary tract in female smokers and non-smokers. Environmental Biotechnology, 10.
Bonassi, S., El-Zein, R., Bolognesi, C. and Fenech, M., 2011. Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. Mutagenesis, 26(1), pp.93-100.
Christobher, S., Periyasamy, M., Nazeer Mohamed, B., Syed Mohamed, H.E. and Sadiq Bukhari, A., 2016 Cytogenetic Biomonitoring: Micronucleus Test in Buccal Epithelial cells of Tobacco Smokers in Tiruchirappalli District (Tamilnadu, India). Int. J. Pharm. Sci. Rev. Res., 38(1),pp:201-2005.
Diler, S.B. and Celik, A., 2011. Cytogenetic biomonitoring of carpet fabric workers using micronucleus frequency, nuclear changes, and the calculation of risk assessment by repair index in exfoliated mucosa cells. DNA and cell biology, 30(10), pp.821-827.
Fenech, M. and Bonassi, S., 2011. The effect of age, gender, diet and lifestyle on DNA damage measured using micronucleus frequency in human peripheral blood lymphocytes. Mutagenesis, 26(1), pp.43-49.
Fenech, M., 2000. The in vitro micronucleus technique. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *455*(1-2), pp.81-95.
Fenech, M., 2007. Cytokinesis-block micronucleus cytome assay. *Nature protocols*, *2*(5), p.1084.
Raj, N.S.S. and Ramdas, A., 2019. micronucleus assay of buccal mucosal cells in smokers and non-smokers. *Indian Journal of Applied Research*, *9*(04).
Schmid, W., 1976. The micronucleus test for cytogenetic analysis. In *Chemical mutagens* (pp. 31-53). Springer, Boston, MA.
Suganya, R., Prabha, V., Lalitha, S. and Rajajeyakumar, M., 2019. The Role of Micronuclei as a Screening Tool in Oral Cancers. *J Clin Exp Patholo*, *9*(368), pp.2161-0681.
Vipul Ja, P.L., Bhanu, A. and Deepak, S., 2017. Buccal cell micronuclei assay: a non-invasive genotoxic marker. *IJCMR*, *4*(1), pp.100-4.
Yee, K.H., Jonarta, A.L. and Tandelilin, R.T., 2015. Micronucleus frequency in exfoliated buccal cells from hairdresser who expose to hair products. *Dental Journal (Majalah Kedokteran Gigi)*, *48*(2), pp.74-79.

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Universitarul Gabriel Corneanu (1942 - 2019) - omul pentru care profesia a fost mai presus de orice

Nu-mi mai amintesc bine când și cum ne-am cunoscut. Se întâmpla cred pe la începutul anilor 1970. Aflasem de la colegii mei de la Stațiunea de cercetări biologice, geologice și geografice ”Stejarul”, Pângărați (Neamț) că trecuse și el, pentru scurt timp, prin acea citadelă a științei din inima Carpaților Orientali, unde făcuse primii pași în cercetare. O părăsise cu doar câteva luni înainte de a descinde eu acolo. Între timp, tânărul om de știință Gabriel Corneanu, căci despre el va fi vorba în cele ce urmează, se mutase cu activitatea în orașul său natal, Craiova, și încerca să se introducă într-un domeniu al biologiei, care în România fusese timp de vreo două decenii deraiat de la cursul lui firesc - Genetica. Era o mare efervescență în jurul acestui domeniu, mulți cercetători tineri doreau să-l îmbrățișeze, poate și din cauză că fusese ani în șir neglijat și hulit pe nedrept. Se făceau eforturi mari și la noi pentru a-l așeza acolo unde îi era locul. Cum la Pângărați, eu și colegul Ion Băra (universitarul de mai târziu), încercam să ne implicăm în acest domeniu de mare perspectivă al biologiei, ni se părea normal să intrăm în contact cu cât mai mulți specialiști din țară care aveau același țel, mai ales cu cei din generația noastră. Între aceștia s-a aflat și viitorul nostru coleg de breaslă și bun prieten Gabi Corneanu. Deși lucram de doar câțiva ani la Pângărați aveam ambiții mari și în vara lui 1972 organizam deja aici prima ediție a unui simpozion pe profil, sub genericul ”*Genetica și progresul social-economic*”. Nu am reușit să reunim atunci la Pângărați decât vreo 30 de tineri cercetători, dar era pentru noi deja un succes, un început promițător.

La a doua ediție a simpozionului, organizată în 1979 la Piatra Neamț și desfășurată pe parcursul a două zile, nu mai eram niște începători în ale cercetării, devenisem între timp doctori în biologie, așa că misiunea noastră de a-i convinge pe unii colegi din țară să ni se alăture la o reuniune de genetică în frumosul oraș de sub Pietricica a fost una mai ușoară. La acea ediție au fost prezenți peste 80 de specialiști din cercetare și din

The academic Gabriel Corneanu (1942 - 2019) - The man who placed profession above it all

I cannot remember exactly when we made our acquaintance. It must have been in the early 1970s. My colleagues from „Stejarul” Station of Biological, Geological and Geographical Research in Pângărați (Neamț) told me that he had started his research in that fortress of science at the heart of the Oriental Carpathians, but had left it just a few months before I got there. In the meantime, the young scientist Gabriel Corneanu, whom I will further present, had moved to his hometown, Craiova, and was trying to introduce a new field of biology - Genetics - which had been taken off its track for about two decades. There was great emulation in this domain, many new young researchers were eager to embrace it, maybe because it had been neglected and unjustly attacked for years. Serious efforts were being made in our country to restore it to its rightful place. As both I and my colleague Ion Băra (the later academic) were making attempts to be involved in this highly promising field, it felt natural to contact as many Romanian specialists as possible, especially the ones of our generation who shared our interest. Among them, we included our colleague and good friend, Gabi Corneanu. Despite our being researchers at Pângărați for only a couple of years, we had the big ambition of organising in 1972 the first edition of a specialised symposium on the theme “*Genetics and social-economic progress*”. We only managed to reunite 30 young researchers, and yet, to us, this was already a success and an encouraging debut.

At the second two-day edition of the symposium, organised in 1979 in Piatra Neamț, we were no longer research “freshmen”, as we had already taken our PhDs in biology, so our mission to convince some colleagues to join us for a genetics reunion in the town at the foot of Pietricica Mountain was successful. In that event, there participated more than 80 specialists from biological, agricultural and medical research and academic institutions from all over the country; among them, there were Gabriel Corneanu and some of his

învățământul superior biologic, agricol și medical, veniți din toată țara, printre care s-a aflat și dr. Gabriel Corneanu împreună cu unii din colegii săi de la Universitatea din Craiova. A fost o ediție foarte specială, mulți dintre participanți apreciind curajul și efortul nostru, importanța acestei reuniuni științifice și a lucrărilor prezentate. Din păcate însă, la următoarele două ediții (în 1981 și 1983) au participat mai puțini specialiști decât în 1979, un motiv fiind probabil și acela că evenimentele științifice pe teme de genetică teoretică și aplicată se înmulțiseră în țară și erau organizate de instituții mult mai puternice decât a noastră.

La ultima ediție a acestui simpozion, colega și prietena mea Gallia Butnaru, profesor la USAMV Timișoara, nu a putut veni la Piatra Neamț, dar s-a gândit să trimită totuși pe cineva care să prezinte lucrarea anunțată în program. Persoana însărcinată cu această misiune se numea Mihaela Cărbunaru, o bănățeancă brunetă, frumușică și dezghețată, studentă în ultimul an, care își elabora teza de diplomă sub îndrumarea doamnei profesor Butnaru. În dimineața deschiderii lucrărilor simpozionului, pe când domnișoara în cauză urca scările ce duceau spre sala de conferințe a Muzeului de Istorie din Piatra Neamț, prima persoană întâlnită în cale a fost domnul Gabriel Corneanu. O întâlnire care avea să-i marcheze pe amândoi și care peste doar patru ani a dat roade, cei doi hotărând să-și unească destinele. Între timp, fosta d-ră Cărbunaru terminase cu brio facultatea și fusese reținută la catedră. Mă consider într-un fel nașul spiritual al familiei Corneanu, pentru că acea ediție a simpozionului amintit a facilitat celor doi ocazia de a se întâlni, a se cunoaște și a constata că au lucruri în comun, ceea ce a dus la constituirea unei familii de universitari de calitate, de care mă leagă o lungă prietenie și frumoase amintiri. Urmărindu-le apoi evoluția profesională, în care maturitatea profesională a lui Gabi s-a îmbinat armonios cu tinerețea și ambiția de a răzbi în cercetare a Mihaelei, am fost bucuros să constat că cei doi se completau reciproc, că munca lor neobosită se concretiza în realizări remarcabile pe plan științific și didactic. Pe parcursul anilor ce au urmat, căutându-i telefonic din diverse motive, constatam adesea că la ore târzii din noapte cei doi se aflau încă la lucru în universitate. Frenezia muncii pusese stăpânire pe ei, iar pasiunea pentru meseria de dascăl și cercetător nu le dădea răgaz. Erau veșnic ocupați și prezenți peste tot: în sălile de curs și de lucrări practice cu studenții și masteranzii, pe teren și în laboratoarele de cercetare pentru realizarea proiectelor contractate, la reuniuni științifice în țară și peste granițe, în biroul de lucru pentru elaborarea de cărți și articole științifice etc.

Unele din preocupările mele științifice și didactice, în special cele de radiobiologie, mutageneză experimentală, biotehnologie și evoluționism, au coincis cu cele ale profesorului Gabriel Corneanu, ceea ce ne-a apropiat și mai mult, ne-a determinat să ne consultăm adesea, să colaborăm în realizarea anumitor proiecte, să ne bazăm unul pe celălalt. Chiar dacă nu ne-am întâlnit atât de des pe cât ne-am fi dorit, ci doar cu ocazia unor reuniuni colleagues from Craiova. It was a special edition, many participants appreciating our efforts and courage as well as the importance of this scientific manifestation and of the papers defended in it. Unfortunately, at the following two editions (1981 and 1983), there were fewer specialists than in 1979, which must have been caused by the proliferation of scientific events in theoretical and applied genetics, held at larger institutions than ours.

At the last edition of this symposium, my colleague and friend Gallia Butnaru, a professor at USAMVB Timișoara, could not come to Piatra Neamț, but she thought of sending someone to present the work announced in the programme. The person in charge of this mission was Mihaela Cărbunaru, a beautiful clever brunette, a student in the last year who was writing her diploma thesis under the guidance of Mrs Butnaru. In the morning of the opening of the symposium's works, while the lady in question was climbing the stairs leading to the conference room of the History Museum in Piatra Neamț, the first person she met was Mr Gabriel Corneanu. A providential meeting which led them, in just four years, to unite their destinies. In the meantime, the former Miss Cărbunaru had successfully graduated with a degree and had become a university assistant. I consider myself, in some way, the spiritual "godfather" of the Corneanu family, because that edition of the symposium facilitated their meeting and knowing each other so as to find that they have things in common and lead to the formation of a family of academics, with whom I have developed a long-lasting friendship and beautiful memories. Following their professional evolution, in which Gabi's professional maturity was harmoniously combined with Mihaela's youthful ambition to succeed in research, I was pleased to find that the two completed each other and their tireless work resulted in remarkable achievements on a scientific and didactic level. During the years that followed, reaching them by telephone for various reasons, I could find them at late hours of the night still working at the university.

They had been totally seized with the frenzy of work and the passion for the teaching and research profession. They were always busy and present everywhere: in the lecture rooms with undergraduate and master's students, in the field and in the research laboratories for the completion of the projects contracted, at scientific meetings in the country and abroad, at the work desk for the elaboration of books and scientific articles, etc. Some of my scientific and didactic interests, especially those in radiobiology, experimental mutagenesis, biotechnology and evolutionism, coincided with those of Professor Gabriel Corneanu, which brought us even closer and often led us to collaborating in the realization of certain projects and relying on each other. Even though we did not meet as often as we wanted to, only at national and international scientific meetings, in committees for PhD public defence or occasionally, we were permanently in contact via phone and mutually supportive. We, together with Professor Ion Băra, had an

științifice naționale și internaționale, în comisiile pentru susținerea publică a unor doctorate sau ocazional, am fost permanent în contact telefonic și ne-am susținut reciproc. Noi doi, împreună cu profesorul Ion Băra, am avut o inițiativă menită să impulsioneze dezvoltarea geneticii în România, unirea forțelor tuturor celor care deserveau domeniul, prin crearea unei Societăți naționale de genetică și a unei reviste de profil. Din păcate, demersul nostru nu a avut ecoul cuvenit în rândul colegilor din țară, așa încât inițiativa a rămas doar în stadiul de proiect. Supărat și dezamăgit de faptul că o inițiativă ca aceasta nu a putut prinde viață, profesorul Băra nu a abandonat cu totul ideea și la sfârșitul anilor 1990 a fondat Seria de *Genetică și biologie moleculară* în cadrul Analelor Universității "Al. I. Cuza" din Iași.

În articolul de față nu mi-am propus să analizez opera profesorului Gabriel Corneanu, contribuțiile sale în domeniile abordate, ci să evoc câteva din coordonatele unui om special, ale unui prieten adevărat, ale unui mare caracter. Nu pot trece totuși cu vederea faptul că unele din abordările sale științifice pot fi considerate lucrări de pionierat în cercetarea românească și nu numai. Iată doar câteva dintre acestea: utilizarea valorii ICV la nucleii interfazici (G_1) pentru stabiliriea valorii D_0 în radiobiologie și a nivelului de ploidie a plantelor; folosirea fluidelor magnetice în stimularea culturilor *in vitro* la plante; utilizarea unor caracteristici ultra-structurale ale nucleului în stabilirea anumitor modificări metabolice normale sau patologice; studiul interacțiunii nanoparticulelor de TiO_2 cu celula eucariotă; evidențierea efectului antistres al unor extracte naturale și produse de origine diversă; impactul unor metale grele și radionuclizi asupra celulei vegetale etc. Nivelul profesional înalt, atins de profesorul Gabriel Corneanu, este atestat de numărul mare de cărți, manuale și articole științifice publicate, de calitatea sa de membru al unor societăți științifice prestigioase din țară și din străinătate, cooptarea lui în comitetele de organizare, științific și de onoare ale unor manifestări științifice, sau în comitetele de redacție ale unor reviste de profil române și străine, în numirea sa ca referent oficial în comisii de susținere ale unor teze de doctorat în diverse centre universitare din țară (el însuși fiind conducător de doctorat în domeniul Biologie-genetică la Univeristatea "Babeș-Bolyai" din Cluj-Napoca), invitația de a susține conferințe și prelegeri la universități și institute de cercetare din Italia, Japonia, Republica Moldova etc. Totodată, printr-o muncă susținută, continuă și deloc facilă, aceea de perfectare și realizare a unor contracte de cercetare de mare valoare în domeniile sale de expertiză, a reușit să atragă fonduri importante, care i-au permis să înființeze noi laboratoare didactice și de cercetare științifică la Universitatea din Craiova și apoi să le îmbunătățească treptat infrastructura de cercetare. A împărtășit din știința și experiența sa și tinerilor studioși de la Universitățile "Ovidius" din Constanța și "Vasile Goldiș" din Arad.

Conștient de valoarea sa, de faptul că numai Dumnezeu le știe pe toate, iar oamenii învață unii de la

initiative to promote the development of genetics in Romania, uniting the forces of all those who served the field, by laying the foundation of a National Society of Genetics and a specialized journal. Unfortunately, our approach did not have the expected echo among colleagues in the country, so the initiative was only in the project stage. Angry and disappointed that an initiative like this could not come to life, Professor Băra did not completely abandon the idea and, in the late 1990s, founded the Series of "Genetics and Molecular Biology" within the Annals of the University "Al. I. Cuza" from Iasi.

In the present article, I do not intend to analyse the work of Professor Gabriel Corneanu, his contributions in the fields addressed, but to evoke some of the coordinates of a special man, of a true friend and of a great character. However, I cannot overlook the fact that some of his scientific approaches can be considered pioneering works in Romanian research. Here are just a few of them: the use of ICV value at the interphase nuclei (G_1) to determine the D_0 value in radiobiology and of the level of plant ploidy; the use of magnetic fluids in stimulating *in vitro* cultures in plants; the use of some ultrastructural characteristics of the nucleus in establishing certain normal or pathological metabolic changes; the study of the interaction of TiO_2 nanoparticles with the eukaryotic cell; highlighting the anti-stress effect of some natural extracts and products of different origin; the impact of heavy metals and radionuclides on the plant cell, etc.

The high professional level, attained by Professor Gabriel Corneanu, is attested by the large number of books, textbooks and scientific articles published, by his membership in prestigious scientific societies at home and abroad, by his participation in the organising and scientific committees of some scientific events, or in the editorial committees of some Romanian and foreign journals, by his appointment as an official referent in the committees for the doctoral theses in various university centres in the country (himself being a doctoral supervisor in the field Genetics-biology at "Babes-Bolyai" University of Cluj-Napoca), and by the invitation to deliver conferences and lectures at universities and research institutes in Italy, Japan, Republic of Moldova, etc. At the same time, through the sustained, continuous and not that easy work of winning and developing high-value research contracts in his fields of expertise, he managed to attract important funds, which allowed him to establish new laboratories of scientific research at the University of Craiova and then gradually improve their research infrastructure. He shared his science and experience with the diligent students from "Ovidius" University of Constanța and "Vasile Goldiș" University from Arad.

Conscious of his own vale and of God only being all-knowing, whereas people are meant to learn from each other, Professor Gabriel Corneanu undertook one action that I greatly admired in him, which few of our academics would practice, namely to invite both

alții, pe când era în activitate profesorul Gabriel Corneanu realiza o acțiune pe care am admirat-o mult la el, pe care puțini dintre universitarii noștri și-au permis s-o practice, și anume să invite colegi din alte centre universitare din țară (între care m-am numărat și eu), dar și personalități ale științei din străinătate (de la universități din Japonia, Italia, Franța, Grecia, Bulgaria) să țină prelegeri de specialitate și conferințe studenților săi. Accepta cu bună știință concurența, dorea ca studenții lui să cunoască și alte modele de urmat, să ia contact și cu alte preocupări și realități, cu alte experiențe de viață și de cercetare, cu alte modalități de exprimare și de prezentare a informațiilor științifice. Nu se temea niciun moment că procedând astfel își va prejudicia prestigiul profesional, că se pune cumva în umbra altora, ci dimpotrivă avea credința că doar așa îi pregătește cum se cuvine pentru viață și profesie pe tinerii săi învățăcei. Câți oare dintre profesorii din învățământul superior al zilelor noastre se încumetă să procedeze ca el?!

O altă calitate umană de invidiat la profesorul Corneanu era francheța. Beneficiind de o experiență importantă în cercetare, de o bună documentare și informare în domeniile de specialitate pe care le acoperea, nu se sfia să spună lucrurilor pe nume în confruntările științifice la care participa. În asemenea ocazii se comporta în stil ”nemțesc”- așa cum ne place nouă să spunem despre un lucru bine făcut. Probabil că multora dintre noi ni s-a întâmplat să asistăm la conferințe, mese rotunde, simpozioane etc, în care s-ar fi impus intervenția cuiva din sală pentru corectarea unor rezultate îndoielnice sau afirmații eronate și totuși nimeni nu a făcut-o. Dintr-un soi de delicatețe prost înțeleasă, sau din dorința de a nu supăra pe cineva, de a nu-și face adversari inutili, oamenii se codesc să ia poziție în unele situații de acest gen. Ei bine, profesorul Corneanu nu făcea parte din această categorie. M-am aflat alături de el la multe reuniuni științifice și m-am convins că participarea lui nu era una de formă, ci una activă, că intervenea în dezbateri ori de câte ori se impunea, venea cu experiența proprie și cu argumente în susținerea sau corectarea unor opinii exprimate, indiferent de persoana în cauză. Pentru el probitatea științifică era un lucru subînțeles, mai presus de orice.

Am admirat și prețuit la profesorul Corneanu simțul solidarității de breaslă și față de prietenii de suflet. Puteai conta pe el când aveai o problemă, indiferent de natura ei. A fost un om deschis, onest și jovial, căruia îi păsa de cei de alături. Mi-a fost dat să-i constat și apreciez prietenia sinceră, respectul și solidaritatea față de mine în cel puțin două ocazii. Arătam anterior că la ultima lui ediție (1983), simpozionul de genetică organizat de noi la Piatra Neamț nu a mai avut ecoul scontat, fiind onorat de mai puțini participanți decât la edițiile anterioare. Întrucât doream să fac din grupul de cercetare pe care îl conduceam unul competitiv și, conștient că prin astfel de acțiuni îți pui în valoare atât potențialul științific cât și capacitatea de organizare, m-am orientat spre o altă temă de interes național. Încă din 1971, din rațiuni financiare, grupul nostru de lucru (redus numeric pe atunci) se axase

colleagues from other Romanian university centres (including myself) and foreign personalities of science (from universities in Japan, Italy, France, Greece, Bulgaria) to deliver specialized lectures and conferences to its students. He willingly accepted the competition, as he wanted his students to have other models to follow, to get in touch with other preoccupations and realities, with other life and research experiences, with other ways of expressing and presenting scientific information. He never feared that, in doing so, he would prejudice his professional prestige, that he would somehow put himself in the shadow of others; on the contrary, he had the strong belief that only this way would he prepare his young students to learn for life and a profession. How many of today's higher education teachers dare to do the same?!

Another enviable human quality possessed by Professor Corneanu was frankness. Benefiting from an important research experience, good documentation and information in the specialized fields that he covered, he would be really outspoken in the scientific confrontations in which he participated. On such occasions he behaved in a "German" style - as we like to say about a well-done thing. Probably, many of us happened to attend conferences, round tables, symposia, etc., where someone in the room should have rejected doubtful results or wrong statements, and yet nobody did. Out of some wrongly understood gentleness or desire not to upset anyone or cause some rivalry, people refrain from taking a stand in such situations. Well, Professor Corneanu would not do that. I participated along with him in many scientific manifestation and I was convinced that his participation was not a formal, but an active one, that he intervened in debates whenever required, came with his own experience and with arguments in support or against some opinions expressed, regardless of the person concerned. For him, scientific probity was by default above it all.

I admired and valued at Professor Corneanu the sense of solidarity of the “guild” and with his soul mates. You could count on it when you had a problem, no matter what its nature. He was an open, honest and joyful man who cared about those around him. I had the chance to acknowledge and appreciate his sincere friendship, respect and solidarity towards me on at least two occasions. We pointed out previously that, at its last edition (1983), the genetics symposium organized by us in Piatra Neamț did not have the expected echo, being honoured by fewer participants than in previous editions. As I wanted to turn the research group that I coordinated into a competitive one and, aware that this is a way to prove one’s value and scientific potential as well as organisational capacity, I turned to another topic of national interest. Since 1971, for financial reasons, our working group (reduced in number at that time) had focused on complex research on some spontaneous and cultivated medicinal plants; meanwhile, we had gained enough experience and some notoriety among specialists in the field, so, in early 1986, I took the initiative to organise a national symposium with the theme "*Medicinal*

pe cercetări complexe asupra unor plante medicinale spontane și cultivate, dobândise între timp destulă experiență și oarecare notorietate printre specialiștii în domeniu, astfel că la începutul lui 1986 am luat inițiativa de a organiza un simpozion național sub genericul ”*Plante medicinale – prezent și perspective*”, prima lui ediție având loc la Piatra Neamț în luna mai a aceluiași an. Simpozionul a stârnit cu fiecare nouă ediție (organizată la interval de 2-3 ani) interesul a tot mai mulți specialiști (biologi, farmaciști agronomi, chimiști), iar după 1990 a căpătat caracter internațional, la lucrările lui participând și specialiști din Republica Moldova, Bulgaria și Serbia. Printre cei prezenți la lucrările acestuia s-au aflat mai de fiecare dată și cei doi prieteni, Mihaela și Gabriel Corneanu, care au apreciat audiența lui, modul de organizare și desfășurare, calitatea lucrărilor prezentate etc. Numai că, așa cum se întâmplă adesea la noi, o colegă pe care o adusesem în grup în 1978, o ajutasem în fel și chip timp de trei decenii, care poza într-o bună ”prietenă”a mea, și-a permis în 2007 să-și însușească calitatea de organizator al acestui simpozion, fără să mă consulte sau anunțe în vreun fel de actul ei ”pirateresc”. În momentul acela mulți dintre cei ce cunoscuseră efortul pe care îl dedicasem ani în șir pentru reușita acestui simpozion au dezavuat gestul ”doamnei”, dar puțini au fost cei care s-au solidarizat cu mine, refuzând să-i onoreze invitația. Printre aceștia din urmă s-a numărat familia profesorilor Corneanu, care i-au declarat franc fostei mele colege că nu puteau cauționa cu prezența lor la simpozion gestul ei necolegial, lipsa de respect și de etică, modul cum procedase față de mine fiindu-le străin.

În toamna lui 2008 m-am confruntat din nou cu un moment neplăcut. Deși eram membru titular al unei Academii din România, calitate care îmi dădea dreptul să profesez până la vârsta de 70 de ani (pentru că așa prevedea legea atunci), deși fusesem până în aprilie rectorul Universității ”V. Alecsandri” din Bacău, cel care a urmat după mine la conducerea universității a decis să-i scoată la pensie pe toți profesorii ce împliniseră vârsta de 65 de ani. Din nefericire, multe universități din țară, sub pretextul unor economii financiare, au căzut în păcatul acesta, renunțând la serviciile unor profesori reputați, de care aveau încă mare nevoie. Surpriza de a fi scos la pensie pe nepusă masă a fost mare nu doar pentru mine, ci și pentru mulți dintre colegii mei, pentru oaspeții ce vizitaseră școala înainte și după mandatul meu de rector și văzuseră schimbarea totală a înfățișării acesteia, îmbunătățirea vizibilă a condițiilor de lucru și de studiu, a vieții în cămine etc. Cine crede că trecerea de la viața activă la cea de pensionar este ușoară și vine ca o binecuvântare după o viață de muncă, se înșeală! Când a aflat această veste, profesorului Corneanu nu i-a venit să creadă și, ca un prieten adevărat ce-mi era, a căutat și găsit cuvinte potrivite pentru a mă ajuta să trec peste acel moment neplăcut, pentru a-mi alunga revolta, dezamăgirea și frustrarea. Numai că, un an mai târziu, avea să fie și el victima unei decizii asemănătoare la Universitatea din Craiova, o decizie la fel de brutală și

plants – current state and perspectives", its first edition taking place in Piatra Neamț in May of the same year.

With each new edition (organised every 2-3 years), the symposium aroused more and more interest on the part of specialists (biologists, agronomist pharmacists, chemists), and, after 1990, it gained international character, given that, in its works, there also participated specialists from the Republic of Moldova, Bulgaria and Serbia. Among those present at almost every edition, we counted the two friends, Mihaela and Gabriel Corneanu, who appreciated the way of organising and managing it, the quality of the works presented, etc. But, as it often happens, a colleague whom I had brought to the group in 1978 and had helped in every possible way for three decades, while posing in a good "friend" of mine, took the liberty in 2007 to assume the status of organiser of this symposium, without consulting me or announcing in any way her "piracy" act. At that time, many of those who had known the effort that I had devoted for years to the success of this symposium disapproved of the "lady's" act, but few were those who sympathized with me, refusing to honour her invitation. Among the latter were the Corneanu family, who declined the invitation to participate and told my former colleague frankly that they could not approve of her non-collegial gesture, disrespect and lack of ethics proven by the way she had treated me.

In the fall of 2008, I faced once again an unpleasant situation. Despite the fact that I was a full member of an Academy in Romania, a position that entitled me to maintain my professorship until the age of 70 (because this was the law at the time) and the fact that I had been the rector of “Vasile Alecsandri” University until April, my successor in this position decided on the retirement of all the professors who had turned 65 years old. Unfortunately, many universities in the country, under the pretext of financial savings, have committed this sin, renouncing the services of reputed professors, who were still in great need. The surprise of being forced to retire was not only great for me, but also for many of my colleagues, for guests who had visited the university before and who had seen after my term as a rector the total change in its appearance, the visible improvement of the conditions for work and study, etc. Whoever thinks that the transition from working life to retirement is easy and comes as a blessing after an active life is wrong! When he heard this news, Professor Corneanu could not believe it and, as a true friend of mine, he searched and found the right words to help me get through that unpleasant moment, to recover from my revolt, disappointment and frustration.

Only that, a year later, he would also be the victim of a similar decision at the University of Craiova, a decision as brutal as it was unexpected given that, for more than four decades, he had served that institution the best he could, placing it on top of his priorities. They disposed of his services without embarrassment, immediately after the specialisation to which he was assigned passed through the periodic evaluation by the ARACIS

neavenită, deși timp de peste patru decenii servise școala aceea cum știuse și putuse mai bine, o pusese mereu în prim planul grijilor sale. Fusese păstrat la catedră până specializarea la care era încadrat a trecut de evaluarea periodică de către comisia ARACIS, după care s-au dispensat fără jenă de serviciile lui. Numai cine trece printr-o astfel de situație poate înțelege cât e de greu. A fost o lovitură neașteptată și dureroasă, care l-a rupt brusc și nemeritat pe Gabi de ce-i era lui mai drag, pe care nu a putut-o uita și depăși, care l-a costat se pare inclusiv sub aspectul stării de sănătate. De data asta am încercat eu să-l consolez cu noul său statut, să-l fac să admită că există viață și după pensie, împărtășindu-i din preocupările care m-au ajutat să mă detașez cumva de acel moment dificil, de o rană ce nu se va vindeca probabil niciodată.

Profesorul Gabriel Corneanu știa să fie o gazdă bună, făcea tot ce-i stătea în putință să te simți bine în preajma lui. Era un tip vesel, atent, generos. Nu te plictiseai alături de el, având mereu ceva interesant de istorisit sau de comentat. Nu pot uita deplasarea întreprinsă cu familia Corneanu la o conferință ce a avut loc în 2007 la Sofia (Bulgaria). Am ajuns cu trenul la Craiova, iar din gară am fost preluat de cei doi și împreună cu ei am mers la un restaurant, iar după cina de acolo am fost cazat într-o cameră de oaspeți a universității. A doua zi dimineață am pornit-o spre Sofia cu mașina condusă de Mihaela. Nu au lipsit unele peripeții pe traseu pentru că, nu-i așa, altfel nu ne-am aminti unele momente trăite în viață. Ajunși la Sofia am beneficiat de o cazare bună, într-un apartament rezervat pentru noi de profesorul Ivan Iliev, prieten al familiei Corneanu. Șederea la Sofia a fost poate unica ocazie în care ne-am aflat mai multe zile împreună, detașați de problemele din țară, un prilej minunat de a discuta nestingheriți subiecte diverse. Mi-a rămas vie în amintire, strădania celor doi de a pregăti micul dejun și cina în bucătăria apartamentului unde eram cazați, grija lor de a mă simți bine, ca și când le-aș fi fost oaspete acasă. Aș zice, fără să exagerez, că s-au purtat cu mine atunci aproape părintește. Atât de grijulii și atenți au fost, încât m-am simțit cu ei ca într-o adevărată familie.

În 2008 am dat curs invitației profesorului Corneanu de a participa la un simpozion organizat la Facultatea de agricultură de la Craiova. De data asta am mers însoțit și de două tinere colege care îmi fuseseră doctorande, la una dintre ele Gabi fiind membru în comisia de referenți. A fost un prilej să cunosc orașul lui natal prin care m-a plimbat, mi-a povestit momente din istoria acestuia, mi-a arătat o serie de clădiri și monumente importante - mândrie a orașului din Bănie, inclusiv una din realizările recente - fântâna muzicală din centrul orașului. Cina festivă a fost organizată într-un adevărat palat, reședința unui fost primar al orașului donată comunității, devenită mai apoi Casa Universitarilor din Craiova. Am fost impresionat de interioarele acestei clădiri, am avut ce privi și admira, inclusiv piesele de mobilier. Acea seară va rămâne și ea un reper al întâlnirilor noastre, pentru că Gabi Corneanu a făcut tot ce putea ca noi, oaspeții lui din Moldova (de la Iași și Bacău), să ne simțim bine. Nu pot

commission. Only those who are faced with such a situation can understand how difficult it is. It was an unexpected and painful blow, which deprived Gabi suddenly and undeservedly of what was dearest to him, a heavy blow which he could not forget and overcome and which impacted on his health. This time, I was the one who tried to comfort him, to make him accept that there is life after retirement, by sharing with him the preoccupations that helped me detach from that difficult moment, from a wound which will probably never heal.

Professor Gabriel Corneanu knew how to be a good host, he was doing everything in his power to make everyone feel good in his company. He was a cheerful, attentive and generous person. One could not get bored with him, as he always had something interesting to tell or comment on. I cannot forget the trip undertaken with the Corneanu family at a conference held in 2007 in Sofia (Bulgaria). I arrived by train to Craiova, and from the station I was taken by the couple to a restaurant, and, after dinner, to a guest room of the university. The next morning we headed for Sofia in Mihaela's car. There were some incidents on the route, of the kind that make us remember some moments lived in life. Arriving in Sofia we benefited from good accommodation, in an apartment booked for us by Professor Ivan Iliev, a friend of the Corneanu family. Staying in Sofia was perhaps our only occasion to spend several days together, away from the problems in the country, a wonderful opportunity to freely discuss various topics. I cherish the memory of the two friends preparing breakfast and dinner in the kitchen of the apartment where we were staying and their care to make me feel good, as if I was a guest at their home. I would say, without exaggeration, that they behaved with me in a parent-like manner. They were so careful and attentive that I really felt like family with them.

In 2008, I honoured the invitation addressed by Professor Corneanu to participate in a symposium organised at the Faculty of Agriculture in Craiova. This time, I went with two young colleagues who had been my PhD students, one of them having Gabi as a member of the committee of referents. It was an opportunity to get to know his hometown, where he took me on a tour, presented important moments in its history, showed me a number of well-known buildings and monuments, including one of the latest achievements – the music fountain at the centre. The festive dinner was organised in a real palace, the residence of a former mayor of the city donated to the community, later become the University House of Craiova. I was impressed by the interiors of this building, I could feast my eyes on everything there, including the furniture. That evening will also remain a milestone of our meetings, because Gabi Corneanu did everything he could for us, his guests from Moldova (from Iasi and Bacau), to feel good. I cannot forget the warmth he showed to my companions whom, to my amusement, he called "little candies". Their reaction is easy to understand! From his height, the professor behaved with the young colleagues from another university centre as a

uita căldura pe care a arătat-o însoțitoarelor mele pe care, spre amuzamentul meu, le-a tratat cu termenul de ”bombonici”. E lesne de înțeles reacția lor! De la înălțimea sa, domnul profesor se comporta cu tinerele colege din alt centru universitar ca un magistru și tutore, dar și ca prieten, încercând să le insufle încredere în potențialul lor și curaj în misiunea asumată, ceea ce nu e puțin lucru la început de drum. Bonomia lui i se citea pe chip, astfel încât una din doctorandele mele, când l-a cunoscut, i-a asemuit figura cu cea a lui Moș Crăciun.

Statutul acesta de gazdă bună și primitoare l-am simțit și cu alte două prilejuri, în orașe în care el însuși era oaspete, dar în care, din motive profesionale sau de familie, se simțea mai acasă decât mine. Gabriel Corneanu își realizase doctoratul în Cluj-Napoca și tot aici devenise peste ani el însuși conducător de doctorate, ceea ce l-a ajutat să cunoască mai bine decât mine orașul, așa încât în 2009 când am fost referent la susținerea tezei uneia din doctorandele sale, s-a ocupat de toate cele ca să nu duc lipsă de nimic, prezentându-mi totodată unele zone și obiective importante din oraș. După ieșirea la pensie profesorul Corneanu a stat mai mult la Timișoara decât în Craiova, întrucât soția sa revenise între timp la universitatea în care se formase (USAVMB), de data asta pe un post de profesor, ocupat prin concurs. La Timișoara ne-am văzut pentru ultima dată. Era în noiembrie 2014 și, profitând de o vizită la Universitatea de Vest într-o misiune de evaluare ARACIS, nu puteam rata ocazia de a-l întâlni pe prietenul meu Gabriel Corneanu. Ne-am plimbat puțin prin oraș, am ”pus țara la cale”, am luat prânzul împreună. De atunci am rămas doar în contact telefonic, uneori și acesta dificil de practicat din cauza stării lui de sănătate. Aș fi dorit să-l revăd în octombrie 2017 când am participat la o conferință AOȘR la Timișoara dar, din păcate, starea de sănătate precară în care se afla nu ne-a permis să ne întâlnim. Am sperat mereu că va depăși momentul greu prin care trecea, că sănătatea i se va ameliora și într-o bună zi ne vom bucura de o revedere specială. Ne-am auzit ultima dată la telefon pe la sfârșitul acestei primăveri. Vocea nu-i trăda starea fizică în care se afla. Părea încrezător că va ieși învingător din lupta cu boala nemiloasă ce-l chinuia de ceva vreme, dar n-a fost să fie! Într-o zi de iulie a acestui an ne-a părăsit pentru totdeauna, lăsând familiei și celor ce i-am fost prieteni un mare gol în suflete, sentimentul neputinței în fața destinului.

Profesorul Gabriel Corneanu va rămâne în memoria noastră o personalitate complexă, un profesionist desăvârșit, un slujitor devotat instituției sale, un soldat credincios misiunii sale de dascăl și cercetător, veșnic la datorie, care a pus mai presus de toate progresul domeniilor științifice în care s-a implicat, un om plin de solicitudine, un tip prietenos, altruist, cald, onest, generos. Pentru mine a fost ca un frate, ceea ce mă face să-i simt și mai acut lipsa. Când ajungi la o anumită vârstă e foarte greu să te desparți de prietenii de o viață! Sper ca Bunul Dumnezeu să-i hărăzească acolo unde a

teacher and a tutor, but also as a friend, trying to instil confidence in their potential and courage in the assumed mission, which is not little at the beginning of one’s career. His being such a good-natured fellow made one of my doctoral students, upon meeting him, liken his figure to that of Santa Claus.

I benefited from this role of a good and welcoming host on other two occasions, in cities where he himself was a guest, but in which, for professional or family reasons, he felt more at home than me. Gabriel Corneanu had obtained his doctorate in Cluj-Napoca and had become a doctoral supervisor himself over the years, which helped him to know the city better than I did, so in 2009, when I was a referent the thesis of one of his doctoral students, he made sure that I would not miss a thing, taking me on a sightseeing tour of the city. After his retirement, Professor Corneanu stayed longer in Timișoara than in Craiova, because his wife had returned to the university where she had graduated (USAVMB), this time as a professor, a position occupied by academic contest. It was in Timisoara that we met for the last time.

In November 2014, taking advantage of a visit to the West University of Timisoara on an ARACIS evaluation mission, I could not miss the opportunity to meet my friend Gabriel Corneanu. We wandered around the city, chatted leisurely and had lunch together. Since then, we have only been in contact by phone, sometimes made difficult by his health condition. I would have liked to see him again in October 2017 when I attended an AOSR conference in Timișoara, but, unfortunately, his precarious state of health did not allow us to meet. I have always hoped that he will overcome the difficult moment he went through, that his health will improve and, one day, we will enjoy a special reunion. We last spoke on the phone at the end of last spring. His voice did not betray his physical condition. He seemed confident that he would win the battle with the ruthless disease that had been tormenting him for some time, but it was not meant to be! On a July day of 2019, he left us forever, leaving behind a large void in the souls of his family and of those who stayed his friends and enhancing the feeling of helplessness in the face of destiny.

Professor Gabriel Corneanu will be cherished as a complex personality, a well-accomplished professional, a devoted servant of his institution, a faithful soldier of the mission of educator and researcher, always on duty, the man who put above all the progress of the scientific fields in which he was involved, a person who was serious, altruistic, honest, generous, warm, friendly and full of solicitude. People like him are met more and more rarely nowadays. For me he was like a brother, which makes me feel even more deeply his loss. When you reach a certain age, it is very difficult to part with lifelong friends! I can only hope that God will bless him, wherever he went, with one place that only He can destine to the chosen ones.

plecat un loc pe care numai EL îl poate rezerva celor aleși.

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