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# Effects of Catholyte Water on the Development of Experimental *Graffi* Tumor on Hamsters

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**Abstract**

The paper describes the results of a pilot study aimed at the investigation of the influence of catholyte (electrolyzed alkaline water) on the development of tumors. In the experiments solid *Graffi* tumor was transplanted subcutaneously in the back of the experimental group of *Golden Syrian* hamsters. Tumor appearance and growth were registered every day. Blood parameters were measured on the 10th and 25th day after transplantation and blood smears were prepared. Hamsters treated with catholyte developed tumor with some delay compared to untreated (drinking tap water) ones. Also, the tumor growth was slow and the survival rate was increased. The analysis of blood parameters and cell morphology has shown significant differences in the value of some hematometric parameters and morphological changes of some blood cells. The obtained results suggest about the beneficial influence of catholyte and the possible use of it as a supporting non-invasive therapy of cancer diseases.

**Keywords:** Graffi tumor, catholyte, tumor growth, survival rate, mortality, blood parameters.

## 1. Introduction

Water is a natural and necessary medium for many biological molecules. Alterations in its composition and structure can produce stimulating or inhibitory effects on the processes in the living things. Influenced by different factors water can change its acidity pH, ORP (Oxidation Reduction Potential), and its physical structure. When electrochemical activation or electrolysis is applied the obtained catholyte receives pH more than 8.0 and negative ORP in milivolts which leads to increased antioxidant effect (Prilutsky, Bakhir, 1997). Due to this reason it could be expected that the catholyte would have protective and positive effect for oxidative stress-related diseases like diabetes and cancer.

Clinical examinations carried out by different scientists have demonstrated positive effect in case of diabetes type 2, telomere shortening in cancer cells and inhibition of their growth, suppression of side effects caused by the use of anticancer medications, favorable influence on the blood system (Hayashi, Kawamura, 2002; Shirahata, 2012; Aschbah, 2019; Gluhchev, Ivanov, 2014; Sanetaka et al., 2012; Ignatov, Gluhchev., 2019). Along with this it was proved that the activated water was not toxic for cells and tissues, and did not have mutagenic, cancerogenic, embryotoxic or immunotoxic effects (Sanetaka et al., 2012).

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For the evaluation of the influence of some medicine or therapy on tumor malignancies different parameters are used based on measurements of tumor development, survival rate, mortality, blood cells changes and others. In the last decade, the main hematometric indices (biomarkers) have been evaluated as diagnostic tools and prognostic parameters in patients with malignancies – cancer and leukemia (Zvetkova, Fuchs, 2017; Hirahara et al., 2016; Azab et al., 2013).

Recent data from the scientific oncological literature evaluated that the NLR (neutrophil to lymphocyte ratio) is superior for predicting the long term survival of cancer patients (Jia et al., 2015; Dezayee, Al-Nimer, 2016; Qin et al., 2018). E.g., lower NLR (≤ 2.0) is associated with good prognosis for breast cancer patients; a higher peripheral blood NLR (≧5) was considered to indicate – significantly and independently, a poor prognosis for breast cancer patients, gastric cancer patients, etc. Simultaneously, the total WBC count, neutrophil and lymphocyte counts alone could be also statistically significant predictors of 5-year cancer patients' mortality (Azab et al., 2013).

At present, the data about the effects of electrolyzed alkaline water (catholyte) on tumor growth and haematological parameters in experimental tumor-bearing animals are absent.

Recently the authors carried out a research about the influence of electromagnetic fields and infrared thermal fields on *Graffi* tumor (Toshkova et al., 2019).

The aim of this study is to examine the biological effects of catholyte on the tumor growth parameters, hematometric biomarkers (including main WBC count; granulocyte and lymphocyte count; lymphocyte percent, GLR (granulocyte to lymphocyte ratio), and blood cell morphology in hamsters with experimental Graffi myeloid tumor.

## 2. Materials and methods

*Experimental animals*. In the trials hamsters, breed "Golden Syrian", aged 2-4 months, male and female, with weight around 100 g, grown in individual plastic cages with free access to food and water were used.

*Experimental tumor*. The experimental *Graffi* solid tumor is maintained on a monthly basis *in vivo* in hamsters from the research team at IEMPAM-BAS (Toshkova, 1995) via subcutaneous **(s.c.)** transplantation of live tumor cells ((1-2.$10^6$) in the area of the back. Between days 7 and 15 in the spot of injection appear tumors, which grow progressively, and the hamsters die aproximately 30-35 days after the injection. In such a tumor model it is observed 100 % appearance (transplantability) of umor and 100 % mortality rate. Spontaneous regression, i.e. spontaneous shrinking and disappearance of the tumor is not observed.

*Catholyte water*. During the experiment catholyte water was produced every day using the Actvator-2 device, developed in the Institute of Information and Communication Technologies at the Bulgarian Academy of Sciences. In this way acidity pH of the water was kept between 9.0 and 9.5 and ORP < 0.

**2.1. Experimental design.** All the animals were divided into 4 groups as follows.

Gr.1 The hamsters from this group started drinking catholyte water 10 days before the injection with 5x$10^4$ *Graffi* tumor cells per hamster in the back area, and continued drinking it until the end of the experiment.

Gr.2. This group was used as a control. The hamsters from it were **s.c.** injected with the same amount of tumor cells on the $10^{th}$ day of the experiment as the hamsters from Gr.1, and were receiving tap water all the time.

Gr.3 consisted of healthy hamsters drinking catholyte water during the experiment.

Gr.4 consisted of healthy hamsters drinking tap water all the time.

The first two groups have to reveal the influence of the catholyte water on the appearance and growth of transplanted tumor compared to the tap water, as well as for evaluation of haematological parameters and peripheral blood cell morphology.

The last two groups were used as control for hematological research.

**2.2. Measured parameters**

The following parameters of tumor development are determined:

- tumor transplantability success (% of hamsters with tumor to the total number of injected ones),
- tumor size (the average diameter of tumor in mm measured with a caliper),
- survival and average survival (calculated for the respective group in days),
- lethality (% of dead animals in the group).

Animals from each group were sacrificed preserving the ethical aspects of the European convention for protection of vertebrate animals, used for experimental and other scientific purposes (OJ L 222), and approved by the National Veterinary Medical Office in Bulgaria, and blood samples have been prepared at different time periods: on days 10 and 25 after *Graffi* tumor implantation. Hematological/hematometric parameters and indices as shown in Fig.1 were measured on the automated hematological analyzer BC-2800 Vet (Mindray, China).

**2.3. The WBC/LR** *(White blood cells to Lymphocyte ratio and NLR (Neutrophil to lymphocyte ratio) were calculated.*

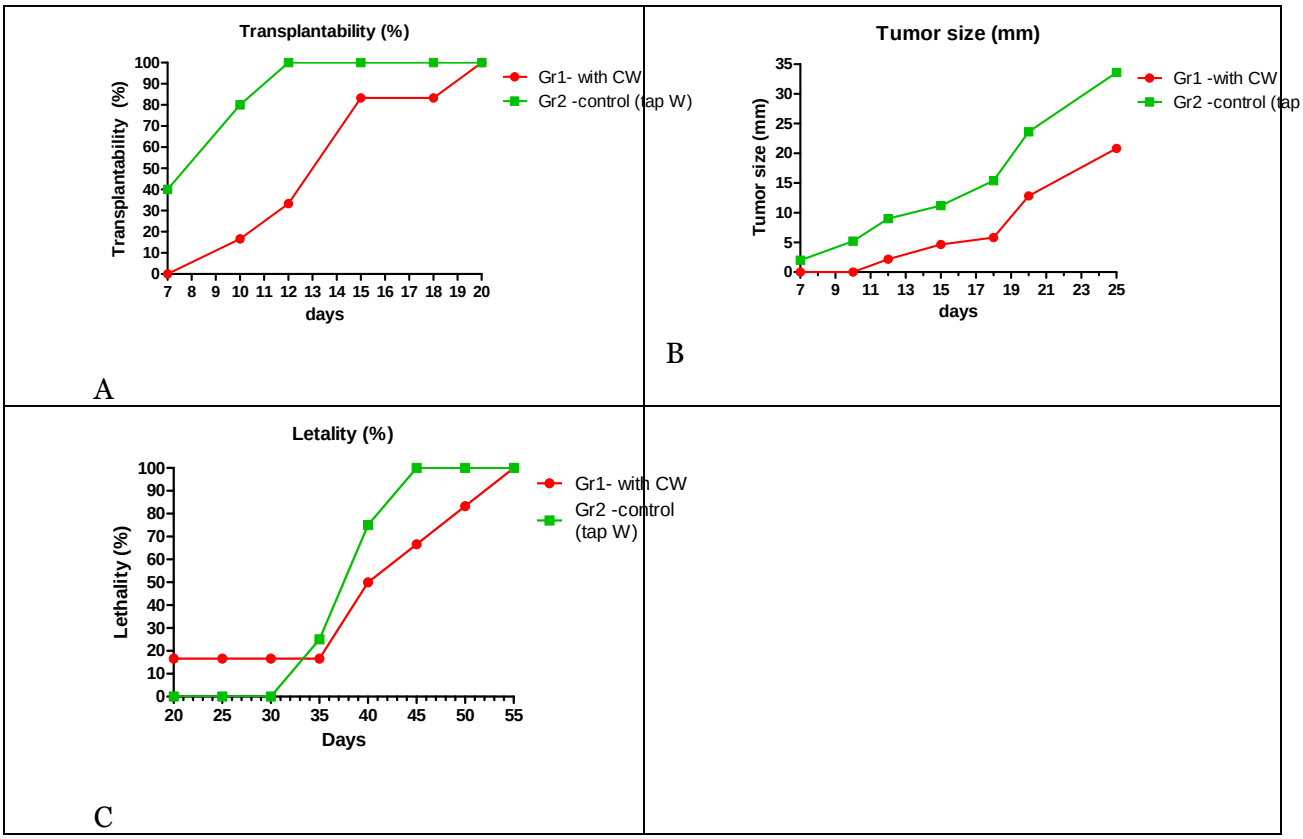
**2.4. *Statistical analysis***

For the evaluation of the significance of the differences between the average values of a specific parameter *t*-test was used at levels of significance α = 0.10 and α = 0.05, respectively.

## 3. Results and discussion

### 3.1. Parameters of tumor growth for Gr.1 and Gr.2.

Catholyte water was used as experimental therapy during two months. Animals have been examined every day until tumor detection and 2 times per week until 30 days after tumor transplantation. The tumor growth parameters have been registered regularly. The differences between the two groups are shown on (Figure 1).



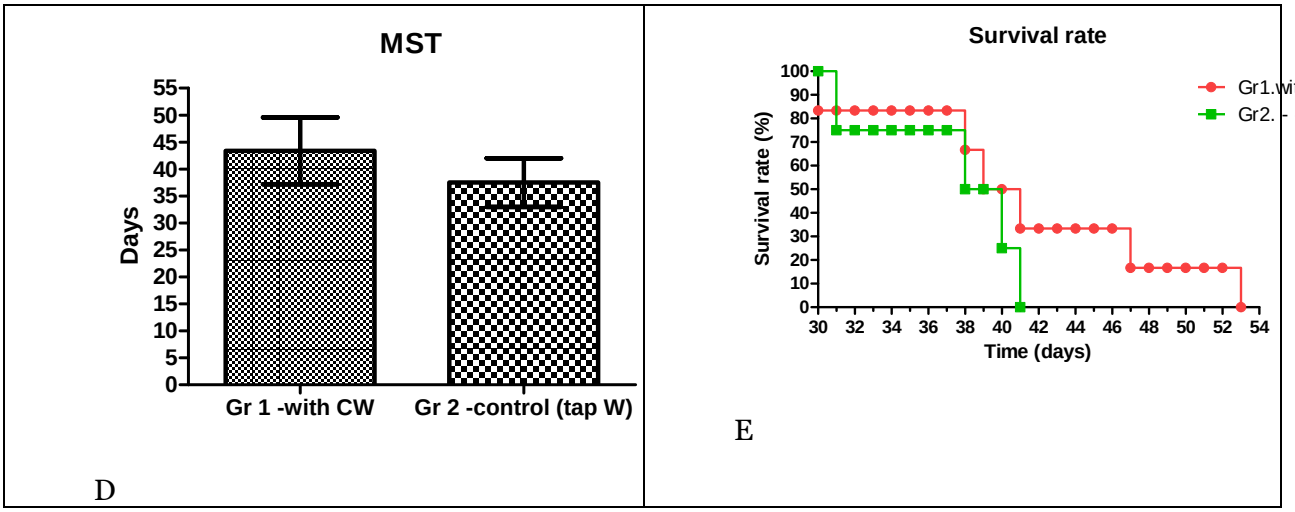



**Fig. 1.** Biometric parameters of tumor growth for the hamsters from Gr.1 and Gr.2: A – transplantability in %; B – tumor size in mm; C – lethality in %; D – average survival time (AST) in days; E – Survival rate

As can be seen from the graph on (Figure 1A) the tumor transplantation was delayed for the hamsters taking catholyte. While all the hamsters from Gr.2 developed tumor on the 12$^{th}$ day this happened only for 1/3 of hamsters from Gr.1. In the hamsters receiving catholite, tumors were detected at 100 % on day 20.

Similar effect is observed for the tumor size (Figure 1B). Until the 10$^{th}$ day subcutaneous firmness in the hamsters drinking catholyte was not established. This group showed an inhibition in tumor growth rate as compared to control throughout the study period.

The lethality for the hamsters from Gr.2 (Figure 1C) is increased after 35 days, compared with the hamsters from Gr.1. The control group (Gr.2) had a 100 % mortality on day 41, while the Gr.1 - on the 53-rd day of the study.

The evaluated average survival for Gr.1 is 43.4±6.9 days, while it is 37.5±4.5 days for Gr.2. Same conclusion could be taken from (Figure 1E).

These data suggest that the catholyte water slows down tumor development and as a result increases survival rate.

The illustrations on (Figure 2) give visual impresion for the tumor development in Gr. 1 and Gr. 2.

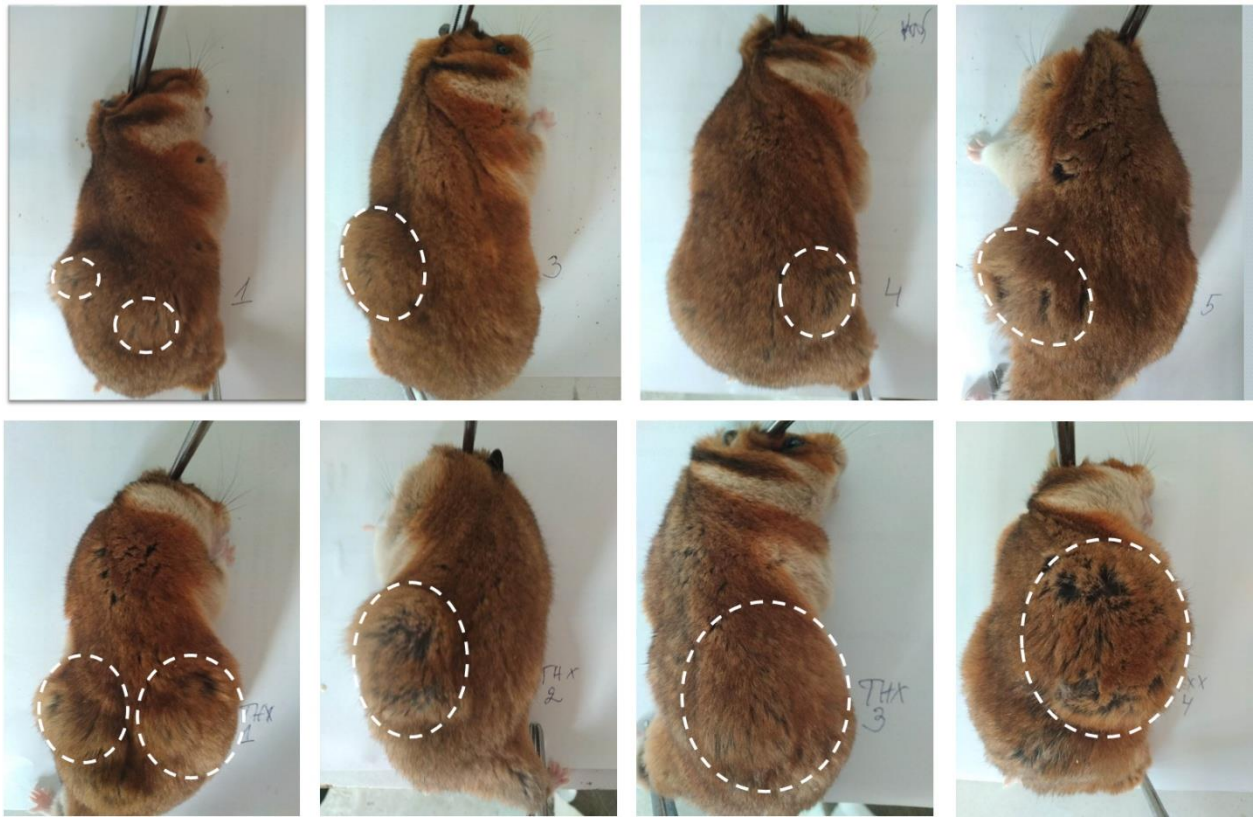


**Fig. 2.** Images of hamsters from Gr.1 (upper line) and Gr.2 (bottom line) taken on the 25th day after the tumor transplantation

### 3.2. Hematological parameters

Hamsters from the trial and control groups were euthanized after the application of deep anaesthesia on the 10th and 25th day after tumor transplanting. The obtained blood was used to report haematological parameters, for serum and preparation of blood smears, as well.

The evaluated parameters for all groups are displayed in (Table 1).

**Table 1.** Blood parameters of 10th/25th day of study

| Parameters | Units | Tumor (Catholyte) | Tumor (Tap water) | Healthy (Catholyte) | Healthy (Tap water) |
|---|---|---|---|---|---|
| WBC (Leukocytes) | $x10^9/L$ | 3.8/ 47.9 | 5.7/2.9 | 6.8/5.8 | 2.4/2.1 |
| Lymph | $x10^9/L$ | 2.2/38.1 | 0.7/0.5 | 5.6/2.1 | 1.4/1.2 |
| Mon | $x10^9/L$ | 0.2/2.3 | 0.5/0.2 | 0.2/0.3 | 0.1/0.1 |
| Gran | $x10^9/L$ | 1.4/ 7.5 | 4.5/2.2 | 1.0/3.4 | 0.9/0.8 |
| Lymph % | % | 58.3/79.5 | 12.8/16.5 | 81.4/35.5 | 58.8/56.3 |
| Mon% | % | 6.0/4.9 | 8.5/7.3 | 3.3/5.2 | 5.7/5.1 |
| Gran% | % | 35.7/ 15.6 | 78.7/76.2 | 15.3/59.3 | 35.5/38.6 |
| RBC (Erythrocytes) | $x10^{12}/L$ | 3.05/2.95 | 4.77/5.58 | 4.7/7.98 | 4.25/5.36 |
| HGB (Haemoglobin) | g/ L | 67/83 | 80/104 | 85/137 | 89/ 92 |
| HCT (Hematocrit) | L/L | 0.165/0.204 | 0.231/0.325 | 0.267/0.445 | 0.226/0.304 |
| MCV (Mean red blood cell volume) | fl | 54.2/69.2 | 48.5/58.3 | 57.0/55.8 | 53.4/56.9 |
| MCH (Average HGB content in erythr) | pg | 21.9/28.1 | 16.7/18.6 | 18.0/17.1 | 20.9/17.1 |
| MCHC(mean conc of Hb | g/ L | 406/406 | 346/320 | 318/307 | 393/302 |
| RDW | % | 12.6/22.9 | 14.1/14.0 | 15/11.7 | 12.8/16.1 |
| PLT(Platelets) | $x10^9/L$ | 132/491 | 883/537 | 250/488 | 306/456 |
| MPV(mean volume of platelets) | f/L | 6.1/7.2 | 4.9/5.6 | 5.4/4.5 | 5.1/5.7 |
| PDW | % | 19.8/19.8 | 17.9/18.3 | 18.9/17.0 | 17.7/19.3 |
| PCT | % | 0.080/ 0.353 | 0.432/.300 | 0.135/0.219 | 0.156/0.259 |

The development of experimental Graffi myloid tumor in hamsters influenced diversely the two main WBC subpopulations – neutrophil granulocytes and lymphocytes (column 4). Significantly elevated WBC, granulocyte count and percent, as well as significant reduction of lymphocyte count and percent were observed in the Graffi myeloid tumor-bearing hamsters (Gr. 2, column 4) and (Figure 3). These effects are well expressed on the 10-th day, and are profound on the 25-th day. The treatment of tumor bearing animals with catholyte as drinking water improved the values of same parameters during the investigation (Gr.1, column 3) and (Figure 3).

Results from the comparison of the blood parameters for which significant difference between Gr.1 and Gr.2 was obtained are shown in (Table 2).

**Table 2.** Significant difference between blood parameters of Gr.1 and Gr.2

| Parameter | Lymp% | Mon% | Gran% | RBC | MCHC | PDW |
|---|---|---|---|---|---|---|
| α | 0.05 | 0.10 | 0.05 | 0.05 | 0.05 | 0.05 |

Based on haematological values the WBCs/LR (White blood cells to Lymphocyte ratio) and NLR (Neutrophil to Lymphocyte ratio) hematometric indices were calculated. Both WBCs/LR and NLR indices are strongly elevated in tumor hamsters taking tap water and highly reduced in hamsters taking catholyte water. The values are similar to healthy hamsters (Figure 3).

Differences in some of the hematological parameters (WBCs, Ly) and WBCs/LR, and NLR hematometric index for groups are shown in (Figure 3).

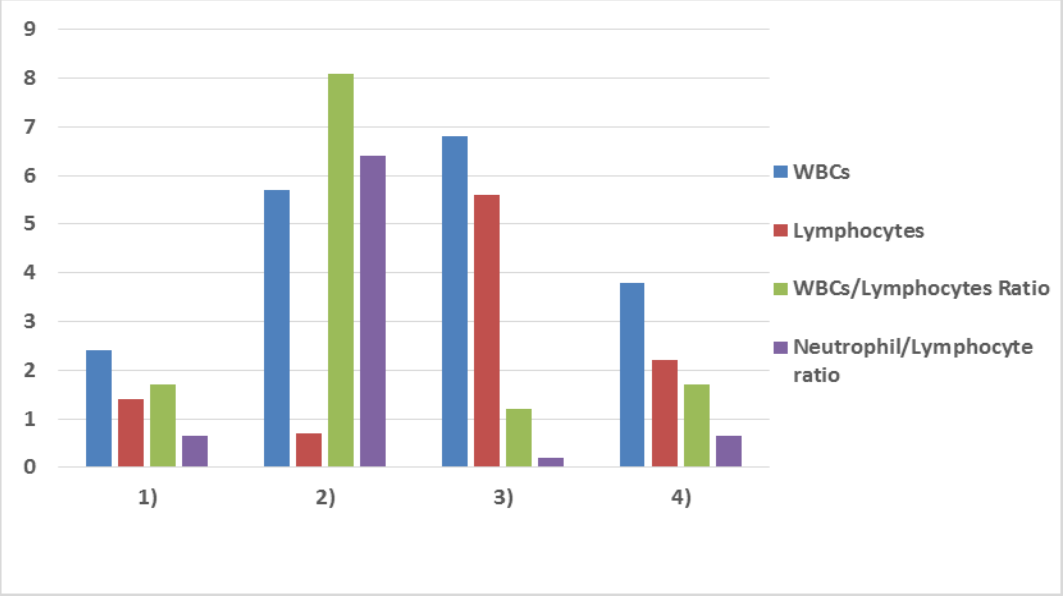



**Fig. 3**. WBCs (leukocytes) and Lymphocytes total count (x $10^9$ /L), and WBCs/Lymphocytes Ratio (at the $10^{th}$ day); 1) Controls; 2) Untreated tumor-bearing hamsters; 3) Healthy hamsters drinking catholyte; 4) Tumor-bearing hamsters drinking catholyte

Significantly elevated WBC count and total granulocyte/neutrophil ratio were obtained in the untreated Graffi myeloid tumor-bearing hamsters (Gr. 2).

Simultaneously, a significant reduction of the lymphocyte number was evaluated in the same animals.

Additionally, we obtained that catolyte water influenced (elevated) some main PLT-hematometric values in both – control and experimental animals (Table 1, PLT, MPV and and PDW). The hematometrical results obtained were confirmed by our cytological studies on PLTs in the peripheral blood smears of hamsters where one could see clusters of activated thrombocytes – more pronounced in the blood of tumor-bearing animals (Fig. 4 -i).

### 3.3. Cytological study

Images from blood smears are shown on (Figure 4).

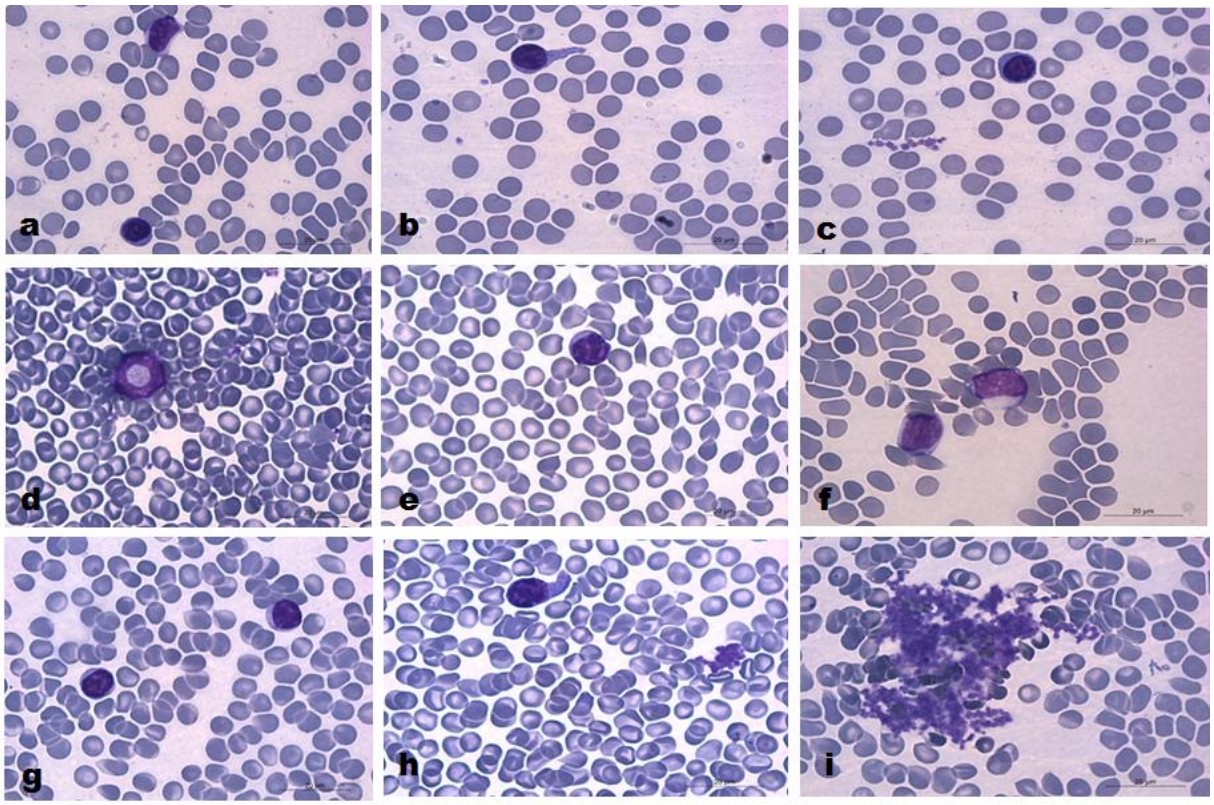



**Fig. 4**. Images of blood smears as follows: upper line - healthy hamster treated with catholyte, middle line – tumor-bearing hamster (control) drinking tap water, and bottom line –tumor – bearing hamster treated with catholyte. (May Gruenwald Giemza staining. Objective X 100).

In the peripheral blood of healthy hamster, taking electrolyzed alkaline water (catholyte) activated lymphocytes with a large cytoplasmic pseudopode (ptotrusion), monocytes and platelets were observed (Figure 4 –a, b, c) upper line.

Atypical myeloid cells and blast-like cells from the peripheral blood of Graffi myeloid tumor-bearing hamsters at the day 10$^{th}$ from tumor inoculation were observed. One could see atypical immature granulocyte with peripherally localized ring-shaped nucleus and eosinophile granules – in the central part of cytoplasm (Figure 4 – d, e, f) middle line.

In Graffi tumor bearing hamsters, treated with catholyte atypical activated lymphocytes and cluster of PLTs in the peripheral blood smear were noticed. Lymphocytes from these hamsters, although atypical, show characteristic signs of activation as in healthy ones (Figure 4 – g, h, i) bottom line.

### 4. Conclusion

The influence of catholyte water on the development of *Graffi* tumor implanted in hamsters was assessed. Some delay in tumor growth and increased survival rate were observed. Significant differences in some of the blood parameters were noticed.

We obtained activated (small and medium-size) lymphocytes in the peripheral blood smears of healthy hamsters – treated with catholyte, instead of tap-water (Figure 4 – a, b, c). The same biological phenomenon was also evaluated partially in the peripheral blood of tumor-bearing animals, under the influence of catholyte (Figure 4-g, h). But in comparison to the activated immunocytes in healthy hamsters, the tumor-infiltration cells (TILs) in the tumor-bearing animals are soon atypical and insufficiently activated (or deactivated – in the preapoptotic or apoptotic states).

The appearance of the so-called blast-like cells (Figure 4 – f) has been related to dissemination of the neoplastic disease and could be earlier obtained in the peripheral blood

smears of untreated tumor-bearing animals (Zvetkova et al., 2006, 2007). The results correlated also with changes in WBCs/LR and NLR hematometric indices obtained in the two experimental groups of treated and untreated tumor-bearing hamsters.

The elevated thrombocytes count could be unfavorable predictor in cancer patients, having in view high risk of thrombogenesis and embolism. On the other hand, the catholyte water could be useful in cases with thrombocytopenia, but not in thrombocytoses, when application of electrolyzed water would be not recommended.

This study also further strengthens the role of WBC-hematometric indices in diagnosis and prognosis of cancer.

Catholyte water (investigated *in vivo* - in our experimental model of Graffi tumor-bearing hamsters), could improve TILs cellular immunity (immunomodulating, immunostimulating influence).

The first conclusion is that the developing experimental Graffi myloid tumor in hamsters influenced diversely the two main WBC subpopulations (predominantly neutrophils) and lymphocytes. These diverse effects – well expressed on the 10$^{th}$ day, are profound on the 25th day.

Our experimental results suggest that in this model the treatment of tumor-bearing animals with catholyte, as drinking water, improves the hematometric indices to the normal values.

Thus, our second conclusion is that the catholyte water – employed instead of tap water in our experimental model with tumor-bearing hamsters, has a positive impact on the main hematometric indices e.g. WBCs/LR and NLR - neutrophil to lymphocyte ratio on day 10 (Table 1) was: for WBCs/LR- 1.71 for a healthy hamster, 1.72 for a tumor-bearing, treated with catholyte and 8.14 for a tumor-bearing, untreated animal, and for NRL - 0.64 for a healthy hamster, 0.64 for a tumor-bearing, treated with catholyte and 6.42 for a tumor-bearing, untreated animal, respectively.

All these points at a favorable influence of catholyte on the hematopoiesis both in case of tumor–bearing animals, and healthy ones.

The obtained results lead to the general conclusion that catholyte could be used as a supporting non-invasive therapy to other cancer therapies as radiotherapy and chemotherapy. However, our pioneer study in this field needs further experimental and clinical confirmation.

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