ORIGINAL ARTICLE

Investigating Neurobion's Dose-Dependent Hematotoxicity in Normal and Neoplastic Human Blood

M. Akhtar[1], M. Shahid[1], F. Siddique[3], H. Ullah[1]

[1] Biophotonics Imaging Techniques Laboratory, Institute of Physics, The Islamia University of Bahawalpur, Pakistan, [2] Department of Computer Science, College of Computer and Information Sciences, King Saud University, Riyadh, Saudi Arabia, [3] Department of Physics, Lahore Garrison University, Lahore, Pakistan.

ABSTRACT

Background: The most prevalent type of cancer in women globally is breast cancer, with an estimated 1.3 million new cases and 465,000 fatalities per year. In 2024, it is projected that approximately 310,720 new cases of invasive breast cancer will be diagnosed in women in the United States. An estimated 42,250 women are expected to die from breast cancer in the same year.
Objectives: the main objective of this work was to extract the variations in shape, size and count of WBCs, RBCs (10^6/L), WBCs (10^3/L), Platelets(10^3/L), HGB(g/dl), RDW(%), PDW(fL), MCV(fL), MPV(fL), HCT(%), and PCT(%) for five concentrations of Neurobion (0 -20) mMol with an interval of 5 mMol.
Materials and Methods: Neurobion has been used as analyte in this experiment. A complete blood count and blood smear of both normal and cancerous blood samples were investigated using optical techniques.
Outcomes/Measures: This manuscript compares the histo-pathological effects of neurobion on the morphology, physiology, and state of blood constituents and parameters.
Sample Size: Three milliliters (3 mL) of blood from both samples were used to assess the comparative health impacts of neurobion.
Results: There was a 1.1×10^3/μL factor increase for normal WBCs and a 0.9×10^3/μL factor increase for cancerous WBCs, reaching optimal levels from their inherent counts. For platelets, there was an 18×10^3/μL factor increase for normal and a 39×10^3/μL factor increase for cancerous samples. RBCs showed a 0.39×10^6/μL factor decrease for cancerous samples.
Conclusions: Neurobion can cause severe negative health effects if used for an extended period.
Keywords: Histopathology, Cobalamin, Thiamin, pyridoxine, Palpation, Mammography

M. Akhtar, M. Shahid, F. Siddique, H. Ullah. Investigating Neurobion's dose-dependent hematotoxicity in normal and neoplastic human blood. Scientific Chronicles 2025; 30(3): 511-521

INTRODUCTION

The way to explain how a device interacts with a specimen and produces images is to say that it either **sends** an electron or light beam through the material in its optical path, **detects** photon emissions from the specimen and/or **scans** across and just a few inches beyond the object being studied [1,2].

Light Microscope

In a digital microscope, instead of looking at the specimen directly through the eyepieces, a digital camera-like sensor is utilized to capture an image that is then

displayed on a computer screen. Depending on the application, such sensors may employ CMOS or charge-coupled device (CCD) hardware. Sensitive photon-counting digital cameras can be used for digital microscopy at extremely low illumination levels to minimize damage to living specimens. A light source that emits pairs of entangled photons has been shown to reduce the risk of harm to samples that are most susceptible to light. This method of using ghost imaging in photon-sparse microscopy involves illuminating the sample with infrared photons, each of which is spatially correlated with an entangled companion in the visible spectrum. This allows effective photography by a photon-counting device, even in the absence of direct illumination of the sample with visible light [3].

To capture photographs of each blood smear, we used a CCD-equipped microscope (Cannon Microscope) made in Japan, which is connected to a computer. The scale bar on this calibrated microscope is 100 µm. By drawing an error bar will allow us to calculate the size of WBCs more accurately. We recorded the photos at a 100-fold magnification. Clear imaging is achieved by using cedar wood oil as an immersion medium.

Breast Cancer.

Breast cancer is the most prevalent type of cancer in women globally, with an estimated 1,300,000 new cases and 465,000 fatalities per year. The age-adjusted incidence rate for breast cancer increased from 36.7 per 100,000 people between 1953 and 1957 to 75.6 per 100,000 people between 2003 and 2007. Early detection and adequate treatment are crucial in lowering breast cancer mortality [4-6]. When a breast tumor is palpable or detectable by mammography, it may have been present for a long time and has had the potential to spread to other distant organs. Breast tumors grow at varying rates depending on the individual. Some tumors develop so quickly that they evade a biannual screening program and exhibit clinical symptoms before a mammography diagnosis. Furthermore, mammography is substantially less sensitive in women with dense breast tissue, a condition commonly found in premenopausal women or in those taking menopausal hormone therapy [7,8]. Other imaging modalities, such as ultra-sonography and magnetic resonance imaging (MRI), have been used in breast cancer screening due to the low sensitivity of mammography in women with dense breast tissue [9]. A convenient alternative modality i.e. light microcopy for diagnostic reasons, blood samples are inexpensive, quickly obtained, and less intrusive.

MATERIAL AND METHODS

Blood samples from both normal and cancerous patients are collected into EDTA tubes via venipuncture. EDTA acts as an anticoagulant. This section will explain three methods for deriving results: (a) complete blood count, (b) blood smear preparation, and (c) microscopy.

1. **Complete Blood Count**: A hematological analyzer named Celltac-α is used to record the variations in blood constituents (WBCs, RBCs, and Platelets) and parameters (HGB, HCT, MCV, MCH, RDW, PDW, and MPV) for all five concentrations of Neurobion: 0 mM, 5 mM, 10 mM, 15 mM, and 20 mM.
2. **Blood Smear Preparation**: A blood smear is performed in three steps: (i) A drop of blood is

spread over a glass slide. (ii) The slide is then allowed to dry, and after drying, the slide is dipped into 80% Ethanol solution, which is called fixing [2]. After fixing, the slide is dried again and then dipped into the field stain (A and B), a process known as staining. A blue stain is used to dye the white blood cells (WBCs), while a red stain is used for the red blood cells (RBCs).

RESULTS AND DISCUSSION

The microscopic and complete blood count results for normal and malignant blood samples are presented in the form of images, tables, and graphs. These results measure the physiological and morphological fluctuations under the five concentrations of neurobion mentioned in Figures 1- 4, as well as in Table 1. This section will explain the variations in blood parameters, blood components, and physiological changes, such as size and shape.

1. Blood components

Blood cells are the components of blood. The state of blood cells, as shown in Table 1 and Figures 1 a-c, illustrates the retarding trend observed under Neurobion for all samples.

Leukocytes: White blood cells (WBCs) serve as the body's defense against germs and diseases by driving and generating the immune response. At the inherent concentration of 0 mM, the WBC count for normal blood was $11.2\times10^3/\mu L$, while for cancerous blood, it was $7.1\times10^3/\mu L$, both of which are considered medically acceptable. At the optimal concentration, the counts were $10.1\times10^3/\mu L$ for normal blood and $6.3\times10^3/\mu L$ for cancerous blood. This represents a factor of retardation of $1.1\times10^3/\mu L$ for normal blood and $0.9\times10^3/\mu L$ for cancerous blood when comparing the inherent to the optimal level. These results indicate an adverse impact of Neurobion on the human immune system.

Thrombocytes: Blood coagulation during cuts, injuries, and diseases is facilitated by platelet cells. The count of thrombocytes varies and shows retarding impacts, as indicated in Table 1. At an inherent concentration of 0 mM, the platelet count for normal blood was $152\times10^3/\mu L$, while for cancerous blood, it was $252\times10^3/\mu L$, both of which are considered medically acceptable. At the optimal concentration, the counts were $134\times10^3/\mu L$ for normal blood and $213\times10^3/\mu L$ for cancerous blood. This represents a factor of retardation of $18\times10^3/\mu L$ for normal blood and $39\times10^3/\mu L$ for cancerous platelet counts when comparing the inherent to the optimal level. These results indicate an adverse impact of Neurobion on the coagulation system.

Erythrocytes: Erythrocytes, also known as red blood cells (RBCs), are responsible for carrying oxygen throughout the body. At an inherent concentration of 0 mM, the RBC count for normal blood was $4.09\times10^6/\mu L$, while for cancerous blood, it was $5.45\times10^6/\mu L$, both of which are considered medically acceptable. At the optimal concentration, the counts were $4.62\times10^6/\mu L$ for normal blood and $5.06\times10^6/\mu L$ for cancerous blood. This represents a factor of attenuation of $0.53\times10^6/\mu L$ for normal blood and a factor of retardation of $0.39\times10^6/\mu L$ for cancerous RBC counts when comparing the inherent to the optimal level. These results indicate an adverse impact of Neurobion on the proper functioning of oxygenated blood

CBC of **normal blood** parameters and constituents											
Sr. No	Neurobion concentration mM	No. of WBCs $10^3/\mu L$	No. of platelet $10^3/\mu L$	No .of RBCs $10^6/\mu L$	HGB g/dL	RDW %	PDW fL	MCV fL	MPV fL	HCT %	PCT %
1	0	11.2	152	4.09	14.5	12.0	17.1	91.0	12.8	44.6	0.19
2	5	10.9	151	4.82	14.2	12.0	17.1	91.5	12.9	44.1	0.20
3	10	10.7	146	4.72	11.3	14.0	21.0	92.6	13.0	43.7	0.19
4	15	10.3	139	4.70	13.8	11.9	22.8	93.0	13.3	43.7	0.18
5	20	10.1	134	4.62	13.6	11.9	16.4	93.5	12.4	43.2	0.17
CBC of **cancerous blood** parameters and constituents											
1	0	7.1	254	5.45	14.6	14.8	14.7	82.9	11.6	45.2	0.30
2	5	7.1	236	5.41	14.4	14.7	15.7	83.5	11.4	45.2	0.27
3	10	6.3	246	5.27	14.1	14.6	14.7	84.8	11.3	44.7	0.28
4	15	6.7	226	5.07	13.8	14.6	14.7	85.6	11.6	43.4	0.26
5	20	6.3	213	5.06	13.5	14.6	15.1	85.6	11.3	43.3	0.24

Table 1. Complete blood count of normal vs cancerous blood under five phantoms of neurobion.

transportation. During any disease or abnormality, the count of blood cells is affected. Blood cells can become dormant during disease. If the analyte is harmful or has an adverse impact on health, then almost all blood constituents and parameters show a retarding trend histopathologically, as shown in Figures 3 (a, b, c).

2. Blood parameters

Blood parameters such as hemoglobin (HGB in g/dL), red cell distribution width (RDW in %), platelet distribution width (PDW in fL), mean corpuscular volume (MCV in fL), mean platelet volume (MPV in fL), hematocrit (HCT in %), and plateletcrit (PCT in %) are vital for the proper functioning of various body systems. Now, we will explain how Neurobion affects HGB, MCV, HCT, and PCV at five distinct concentrations: 0mM, 5mM, 10mM, 15mM, and 20mM.

Hemoglobin (HGB): The value of hemoglobin (HGB) is 14.5 g/dL without Neurobion, or at an inherent concentration of 0 mM. However,

it drops to 14.2 g/dL when the Neurobion concentration is increased to 5 mM. HGB has a typical range of 11.5-16.5 g/dL. As we add more Neurobion up to 10 mM, the value of HGB rises to 14.3 g/dL. At 15 mM, HGB is 13.8 g/dL, and at 20 mM, it is 13.6 g/dL, indicating that Neurobion levels are at their optimal levels. Therefore, we can detect a decrease in HGB for normal blood of up to 0.9 g/dL when the Neurobion concentration is increased from its inherent to its optimal value. In malignant blood, HGB has a value of 14.6 g/dL at an inherent concentration of 0 mM. However, when the concentration of Neurobion is increased to 5 mM, the value of HGB drops to 14.4 g/dL. As we continue to increase Neurobion up to 10 mM, the value of HGB decreases to 14.1 g/dL. At 15 mM, HGB is 13.8 g/dL, and at 20 mM, it is 13.5 g/dL. As a result, we have observed a decrease in HGB of up to 1.1 g/dL when the Neurobion concentration in malignant blood is increased from its inherent to its optimal value. Mean **Cumulative Volume (MCV):** When Neurobion is added at a concentration of 5 mM, the mean corpuscular volume (MCV) value rises to 91.5 fL, falling within the normal range of 76-96 fL. Normal blood has an inherent MCV level of 91.0 fL. However, if the Neurobion concentration is raised to 10 mM, the MCV value increases to 92.6 fL. As more Neurobion is added, up to 15 mM, the value of MCV climbs and reaches 93.0 fL. At a concentration of 20 mM Neurobion, MCV is 93.5 fL. We can therefore see 2.5 fL attenuation in the MCV level from the inherent to the optimal Neurobion content. In contrast, the MCV value for malignant blood increases to 83.5 fL when Neurobion is added at a concentration of 5 mM, from an inherent value of 82.9 fL. If the Neurobion concentration is raised to 10 mM, the MCV value rises to 84.8 fL. As we add more Neurobion up to 15 mM, the value of MCV climbs and reaches 85.6 fL. At 20 mM Neurobion, the value of MCV remains at 85.6 fL. The attenuation of the MCV level is therefore 2.7 fL from the inherent to the optimal Neurobion content.

Hematocrit (HCT): When Neurobion is added at a concentration of 5 mM, the hematocrit (HCT) value drops to 44.1% from its inherent value of 44.6% in normal blood. However, the HCT value decreases to 43.7% if the Neurobion concentration is increased to 10 mM. As we add more Neurobion, up to 15 mM, the HCT value remains at 43.7%. At a concentration of 20 mM Neurobion, the HCT value drops to 43.1%. Therefore, we observe a 1.5% decrease in HCT from the inherent to the optimal

Neurobion content: In contrast, the HCT value for malignant blood is 45.2% at the inherent Neurobion level and falls to 45.0% when Neurobion is administered at a dose of 5 mM. However, the HCT value drops to 44.7% if the Neurobion concentration is increased to 10 mM. As we add more Neurobion up to 15 mM, the HCT value drops to 43.4%. At 20 mM Neurobion, the HCT value is 43.3%. Therefore, we can see a 1.9% decrease in HCT from the inherent to the optimal Neurobion content. The remaining haematological variables, such as red cell distribution width (RDW), mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT), also exhibit slight decreases from the intrinsic to the optimal level of Neurobion.

3. Physiology of WBCs

The size and shape of normal and malignant leukocytes have been analyzed

under the aforementioned five concentrations of neurobion, as detailed below.

3.1. Shape abnormalities:

Leukocytes display variations from their normal form in normal blood. At concentrations of 10 mM to 15 mM, leukocytes change shape from irregular to a regular round form. At the optimal concentration of 20 mM, leukocytes swell, and their outer membrane appears ruptured. WBCs, which typically have an almost round shape, transform from round to irregular, as shown in Figure 1 (a-e).

In cancerous blood, leukocytes are rounded, as seen in figure 2 (a). At concentrations of 5 mM and 10 mM neurobion, leukocytes change shape from round to elongated, as shown in figures 2 (b, c). From 15 mM to 20 mM neurobion, they transition from elongated to swollen elliptical shapes, as seen in figures 2 (d, e). Thus, both benign and malignant leukocytes exhibit negative shape effects due to neurobion.

3.2. Size attenuation:

Leukocytes have a permeable cell membrane, and diffusible analytes not only disturb the proper function of WBCs but also affect their physiology, including shape and size. Using optical microscopy with a scale bar with 10 µm calibration, we can measure the size of leukocytes using Image J. For normal blood, the size of WBCs at 0 mM is 5.77 µm, which increases to 9.68 µm at the optimal concentration of 20 mM, resulting in a 3.91 µm increase in size as we mixed Neurobion from its inherent concentration (0 mM) to the optimal concentration (20 mM) in 5 mM increments. In contrast, malignant leukocytes exhibit a greater increase in size compared to normal leukocytes. For cancerous blood, the size of WBCs at 0 mM is 5.42 µm, increasing to 10.11 µm at 20 mM, resulting in a 4.69 µm increase in size (Table 2). This suggests that the count of leukocytes decreases because, as neurobion enters the cells, they begin to swell. Each cell has an elastic limit, and when this limit is exceeded, the cells start to burst. Therefore, the size of the cell is inversely proportional to the count of cells under the influence of Neurobion.

CONCLUSION

Under five concentrations of neurobion (0 mM, 5 mM, 10 mM, 15 mM, and 20 mM), various blood parameters exhibit changes:

i. Red blood cells (RBCs in 10^6/L), white blood cells (WBCs in 10^3/L), platelets (10^3/L), hemoglobin (HGB in g/dL), red cell distribution width (RDW in %), platelet distribution width (PDW in fL), mean platelet volume (MPV in fL), hematocrit (HCT in %), and plateletcrit (PCT in %) show **retardation**.

ii. Mean corpuscular volume (MCV) exhibits **attenuation**.

iii. Leukocyte physiology, including the shape of WBCs, is also impacted. Both benign and malignant leukocytes display **adverse shape effects** from neurobion.

iv. With increasing neurobion concentration, the **size of WBCs** distinguishes between benign and malignant cases.

v. Under the influence of Neurobion, the **relationship between the cell counts and the cell size is inverse**. In conclusion, Neurobion causes very serious negative health effects.

Limitations

This is a two-dimensional analysis and cannot provide the three-dimensional morphology of cells, which can be assessed using laser scanning microscope.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Sr. No	Molar concentration (mM)	Size of WBCs of Normal blood (µm)	Size of WBCs of Normal blood (µm)
1	0	5.77	5.55,4.67,5.06.44mean=5.42
2	5	8.66,7.46,7.46,8.95 Mean = 8.13	8.35
3	10	8.14	9.71
4	15	9.67	7.81, 5.16 mean= 6.48
5	20	9.68	10.11

Table 2. Describing the size variations of WBCs of normal vs. Cancerous blood under Neurobion.

FIGURES

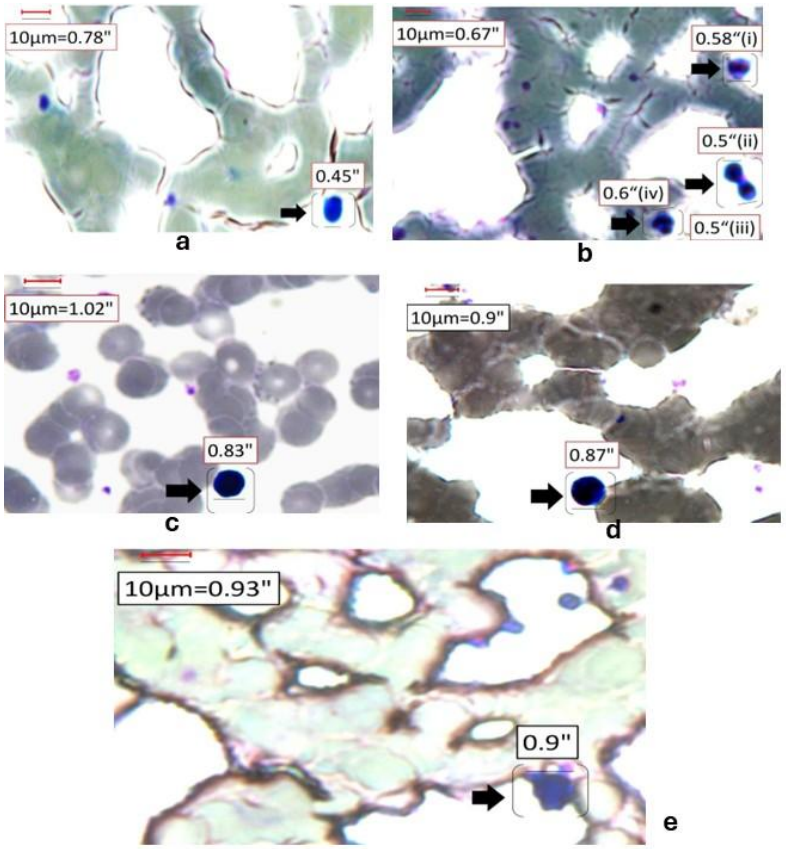

Figure1 (a-e). Depicting the physiological variations of WBCs of normal blood for each phantom of Neurobion using an optical microscope at 100X.

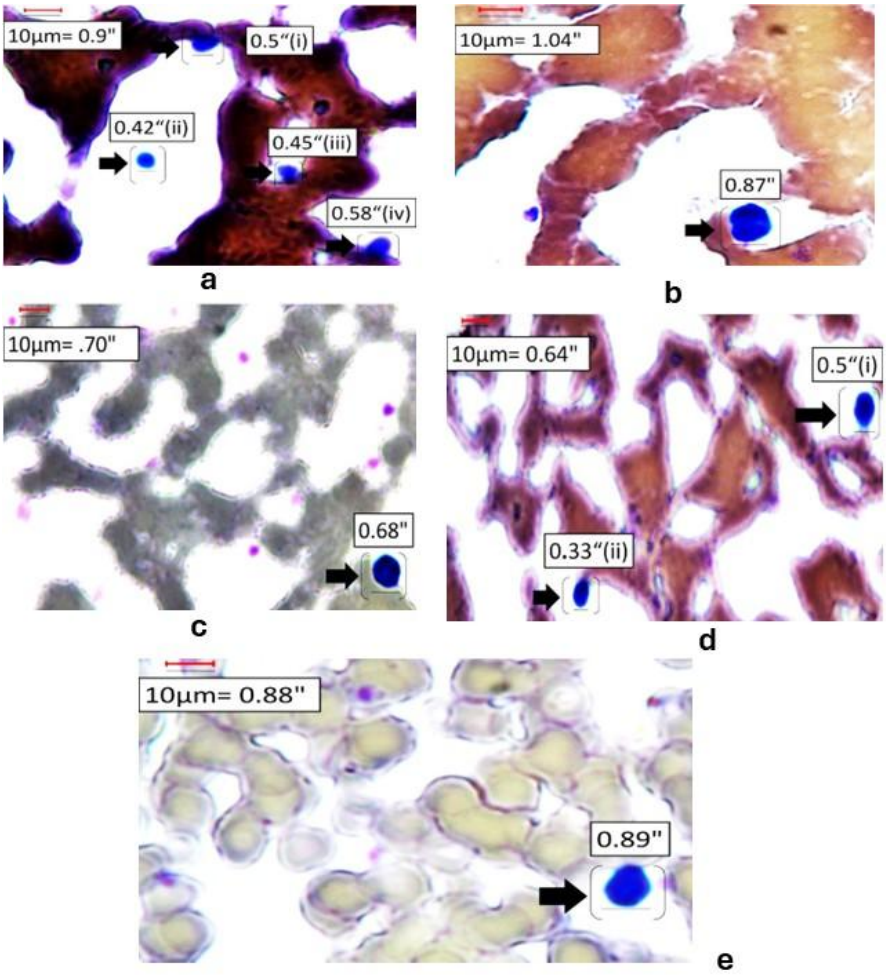

Figure 2(a-e). Depicting the physiological variations of WBCs of Cancerous blood for each phantom of neurobion using optical microscope at 100X.

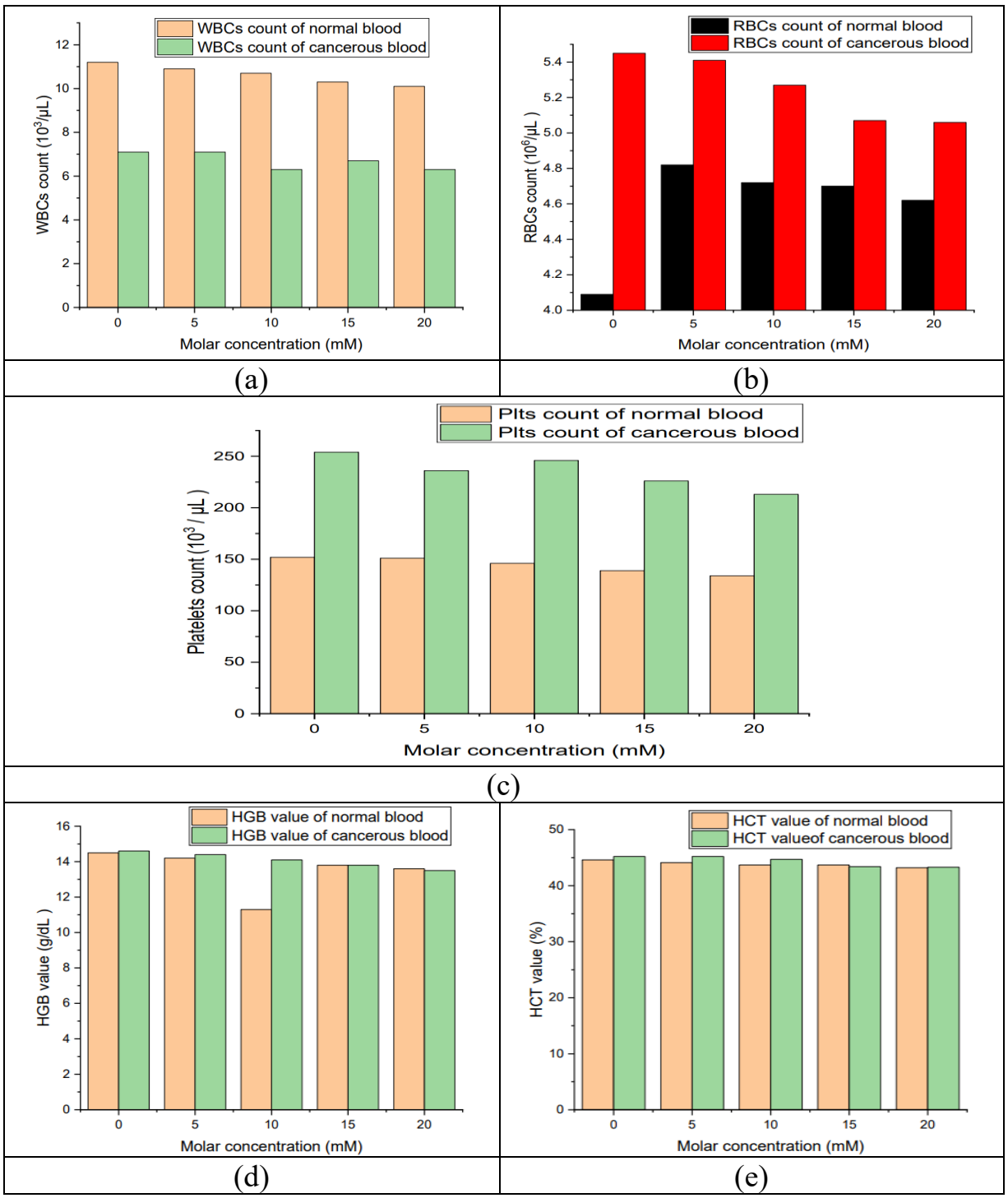

Figure 3 (a-e). Showing decreasing trend of WBCs, RBCs, Platelets, HGB and HCT under increasing concentrations of Neurobion.

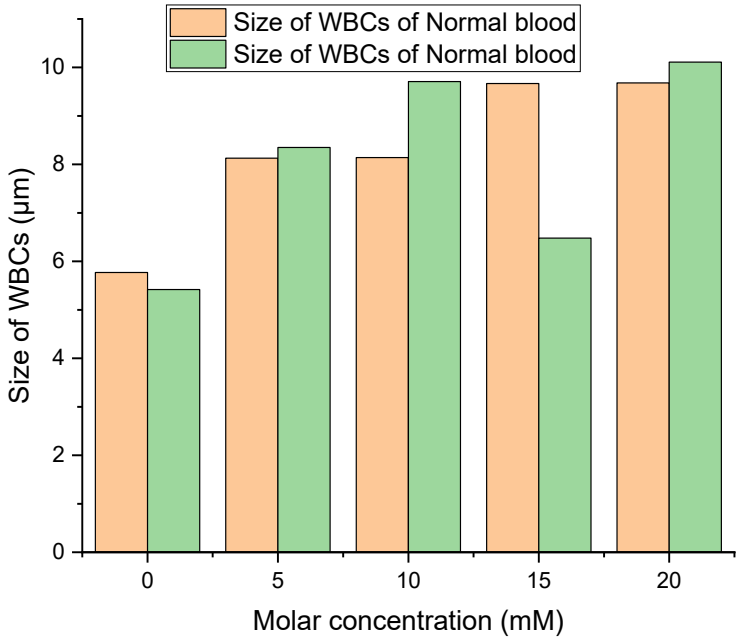

Figure 4. Elaborating the size variations of WBCs of each blood for each phantom of neurobion.

REFERENCES

1. Liu H. Using Tunable Lens to Extend the Range of the Single-Particle Tracking Microscop, 2018, The Ohio State University.

2. Zempleni J, Suttie JW, Gregory III JF, Stover, PJ. Handbook of vitamins. 2013: CRC Press.

3. R. Aspden, N. Gemmell, P. Morris, D. Tasca, L. Mertens, M. Tanner, et al. Photon-sparse microscopy: visible light imaging using infrared illumination. Optica 2015; 2: 1049-1052.

4. Zell JA, Cinar P, Mobasher M, Ziogas A, Meyskens FL Jr, Anton-Culver H. Survival for patients with invasive cutaneous melanoma among ethnic groups: the effects of socioeconomic status and treatment. J Clin Oncol. 2008 Jan 1;26(1):66-75.

5. Larsen IK, Småstuen M, Johannesen TB, Langmark F, Parkin DM, Bray F, Møller B. Data quality at the Cancer Registry of Norway: an overview of comparability, completeness, validity and timeliness. Eur J Cancer. 2009 May;45(7):1218-1231.

6. Feigelson HS, Henderson BE, Pike MC. Re: Recent trends in U.S. breast cancer incidence, survival, and mortality rates. J Natl Cancer Inst. 1997 Dec 3;89(23):1810-2.

7. Weedon-Fekjaer H, Lindqvist BH, Vatten LJ, Aalen OO, Tretli S. Breast cancer tumor growth estimated through mammography screening data. Breast Cancer Res. 2008;10(3):R4

8. Kolb TM, Lichy J, Newhouse JH. Comparison of the performance of screening mammography, physical examination, and breast US and evaluation of factors that influence them: an analysis of 27,825 patient evaluations. Radiology. 2002 Oct;225(1):165-75.

9. Weedon-Fekjaer H, Lindqvist BH, Vatten LJ, Aalen OO, Tretli S. Breast cancer tumor growth estimated through mammography screening data. Breast Cancer Res. 2008;10(3):R41.

Corresponding author: **Fatemeh Bezaatpour,** E-mail: bezaatpourfatemeh@yahoo.com