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Hematological complications under hyper conditions of glucose and distilled water

Hafeez Ullah[1*], Munir Akhtar[2], Fayyaz Hussain[2], Ejaz Ahmad[2], Mukhtar Ahmad[3], Muhammad Hammad Aziz[3], Farah Andleeb[4] and Zahida Batool[1]

[1]Bio-Photonics Research Laboratory Department of Physics, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[2]Department of Physics, Bahauddin Zakariya University, University Campus, Bosan Road, Multan, Pakistan
[3]Department of Physics, COMSATS Institute of Information and Technology, Lahore, Pakistan
[1,4]Department of Physics, Govt. Sadiq College Women University, Bahawalpur, Pakistan

Abstract: This work presents a pilot method of hematological diagnosis about changes in: shape, size and rouleaux formation, cell count of leucocytes and platelet cells in the presence of different glucose [$C_6H_{12}O_6$] and water [H_2O] concentrations. The 2D microscopic images after addition of ten different glucose concentrations to normal blood (0 mM- 450 mM) revealed the lyses (disintegration) of white blood cells (WBCs). This work provides a baseline to diagnose blood disorders and complications at labs and clinical environment.

Keywords: Glycolysis, hyperglycemia, glucometery, CBC (complete blood count).

INTRODUCTION

Blood is a fluid in our body which is not only responsible for transportation of nutrients within the body (proteins, fats, minerals and carbohydrates, etc.), but also waste materials (e.g. carbon-dioxide) out of body (Krieglstein *et al*., 2007). Usually 2-3 drops of blood contains 1 billion red blood cells (RBCs) and for every 600 RBCs there are only 1 WBC and 40 platelets (Ross *et al*., 1988). Normal glucose level after fasting is typically between 70 and 99mg/dL and 2 hours after eating a meal is less than 140mg/dL (Engelgau *et al*., 2000). Hyperglycemia is a condition where the blood contains more glucose than normal, and hypoglycemia, the blood contains less glucose than normal (Sperelakis, 2012).

We have investigated three parameters of leucocytes i.e. (*i*) shape change, (*ii*) change in size and (*iii*) cell count under hyperglycemia and hypotonic solution of distilled water by using optical microscopy. The most prominent feature is that it does not use harmful ionizing radiation. In this work, we have used white light and dark field digital microscopy along with a CCD camera to record images in transmission mode.

MATERIALS AND METHODS

In this work, we have investigated two types of samples to diagnose blood parameters and blood cells.

Sample set (I)

Our sample set (I) consists of leucocytes with ten different concentrations (0mM, 50mM, 100mM, 150mM, 200mM, 250mM, 300mM, 350mM, 400mM and 450mM of glucose and distilled water. We took 10 samples of 2 ml of fresh blood for glucose and 4 ml for distilled water from healthy volunteer (blood type AB+) by vein puncturing. Samples were stored in Ethylenediaminetetra acetic acid (EDTA) tubes. We added the aforementioned concentrations of glucose and distilled water in each heparin tube 0 mM means normal blood i.e. no extra glucose or water addition. We used ethyl alcohol for fixing and field stain (A, B) for staining. Slides for each concentration were then examined using a white light microscope (Olympus CX41) at 100X after putting one drop of cedar wood emersion oil. This oil enhances the capability of lens. The resultant changes in shape and size of leucocytes were then captured using a CCD (Canon EOS 600D, Japan). The experiment was performed at room temperature (21^{o}C).

Sample set (II)

Our sample set (II) consists of platelets obtained by centrifuging the blood from same healthy male volunteer by vein puncturing. The blood was taken into ten Ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes. Each tube contained 2 ml of blood for sugar and 4ml for water analyte. We mixed the nine different measured concentrations into nine different EDTA tubes.

Ethical approval

It is stated that all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Corresponding author:* e-mail: hafeezullah79@gmail.com

STATISTICAL ANALYSIS

We have used Microsoft excel 2010 for the statistical analysis of all the parameters and findings are noted by mean, average and standard deviation.

RESULTS

Glucose's effect on blood components

2D images with white light microscope of sample set (I) for different concentrations of 0mM, 150mM, 250mM and 450mM of glucose analyte captured using CCD are shown in fig. 1 for illustration purpose. 2D images of dark field microscopy of sample set (II) for concentrations of 0mM, 150mM, 250mM and 450mM of glucose analyte are shown in fig. 2 for illustration purpose.

Fig. 1 (a) shows the normal shape; fig. 1(b) at 150mM (54 mg) demonstrates a spiral shape being generated. Fig. 1 (c) at 250 mM (90mg) concentration of glucose shows a small amount of elongation and fig.1(d) at 450 mM (162mg) concentration of glucose demonstrates a trend

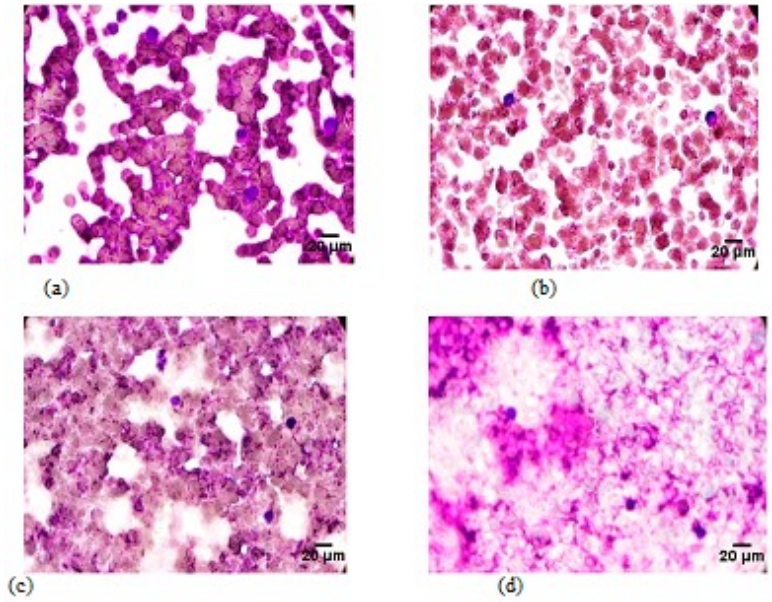

Fig. 1: Light microscopic images forWBCs for (a), 0 mM concentration of glucose, (b) 150 mM, (c) 250 mM, and (d) 450 mM concentration of glucose for illustration purposes.

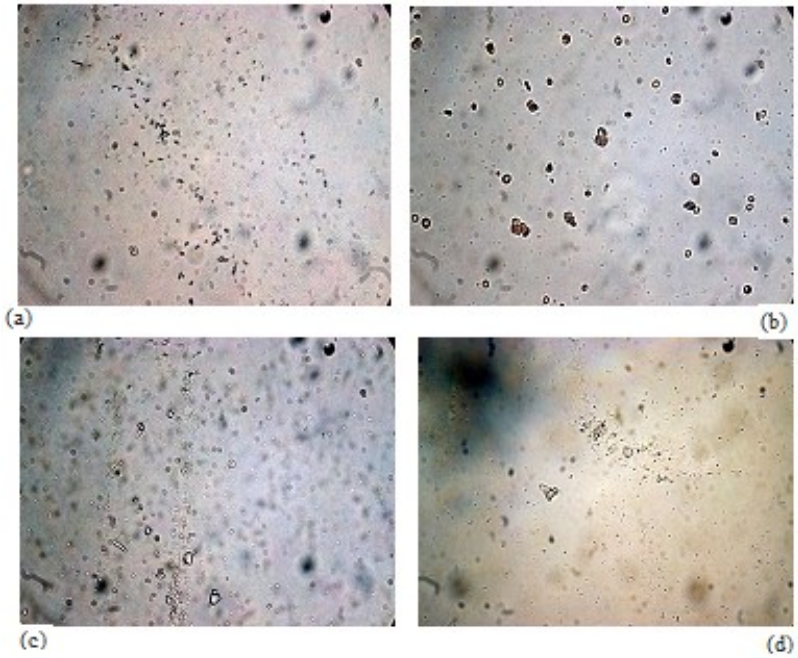

Fig. 2: Dark field microscopic images of PRP sample for (a) 0 mM concentration of glucose, (b) 150 mM, (c) 250 mM and (d) 450 mM concentration of glucose for illustartion purposes only.

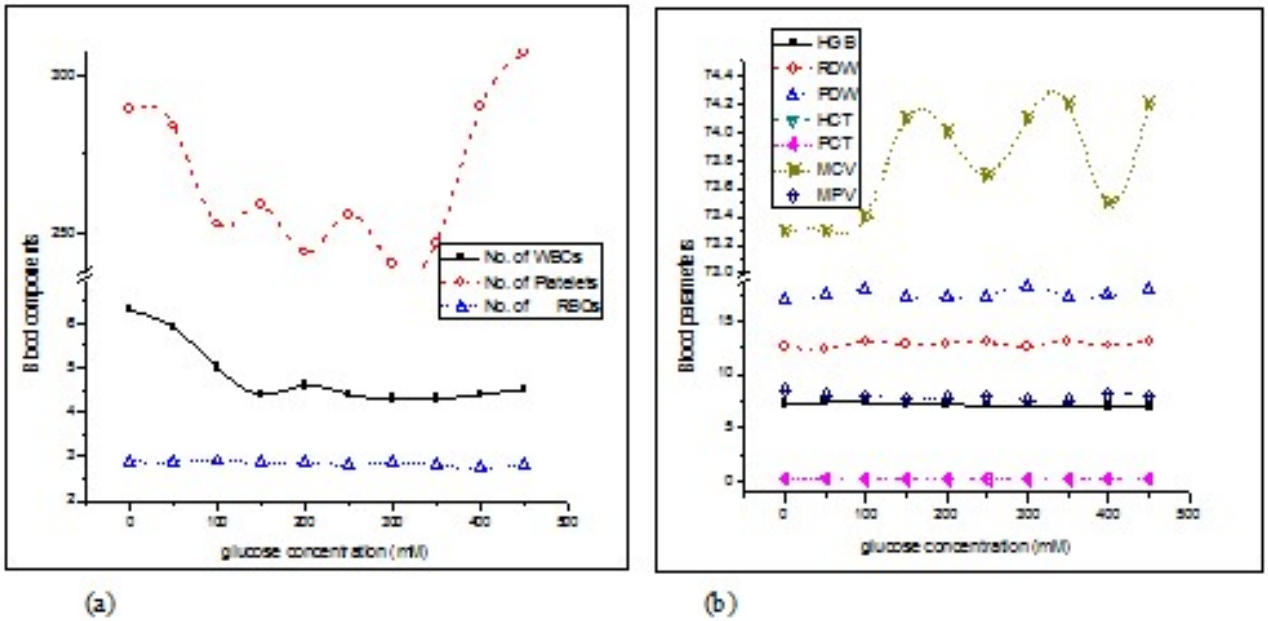

Fig. 3: (a) Glucose concentrations versus blood cells, and (b) glucose concentrations versus blood parameters. The appropriate effect of glucose concentration can be observed on MCV parameter.

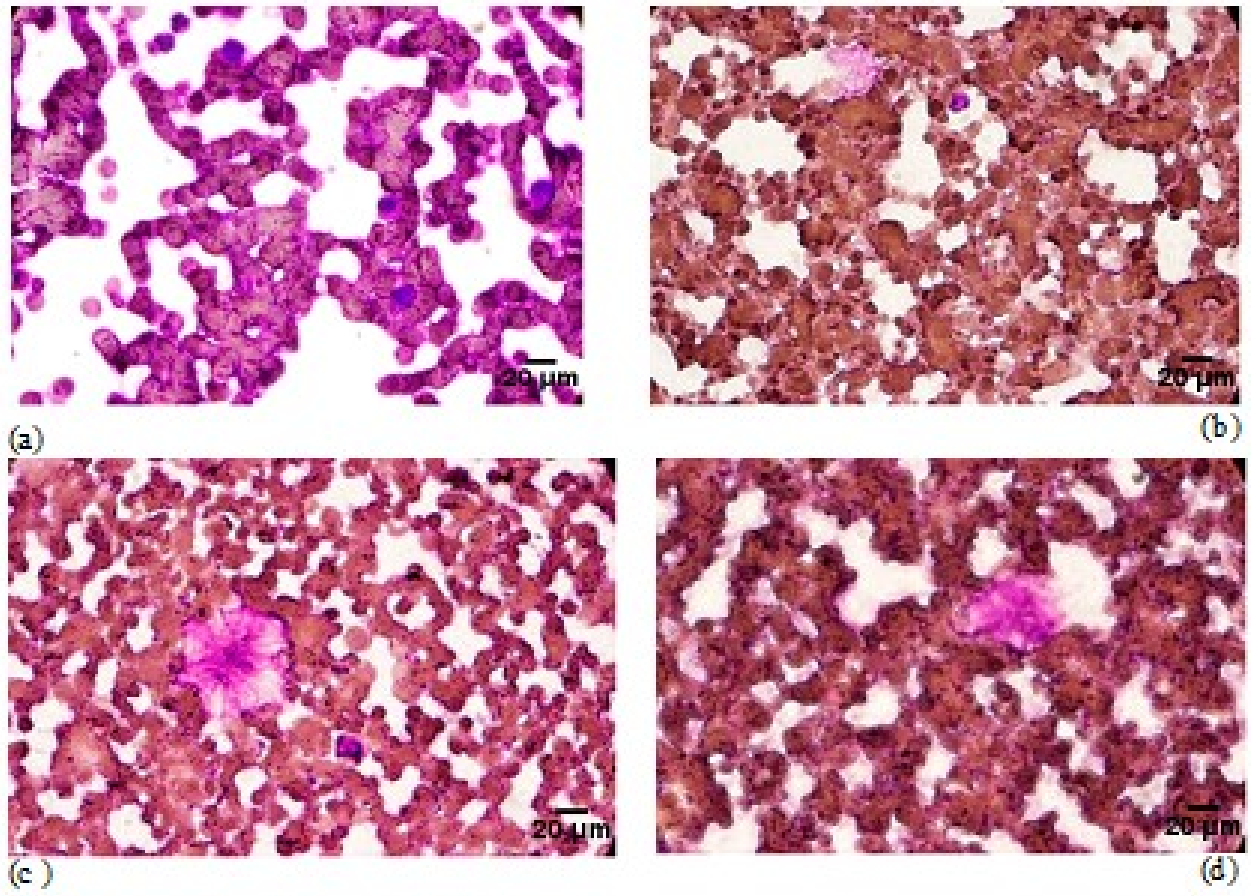

Fig. 4: Light microscopic images of whole blood sample for (a), 0 mM concentration of water, (b) 250 mM, (c) 300 mM and (d) 350 mM for illustration purposes.

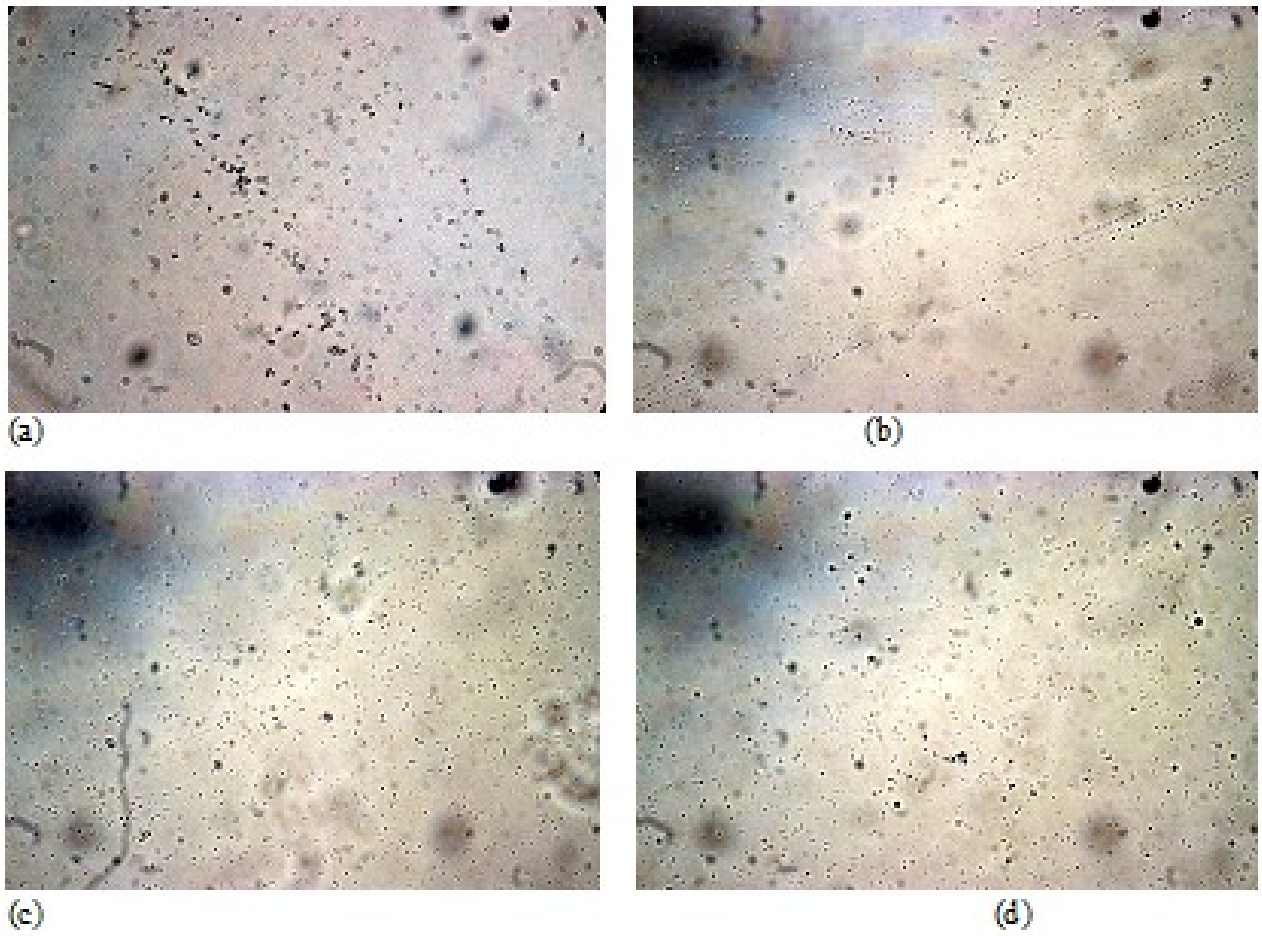

Fig. 5: Dark field microscopic images of PRP sample for (a) 0 mM concentration of water, (b) 200 mM, (c) 400 mM, and (d) 450 mM illustartion purposes only.

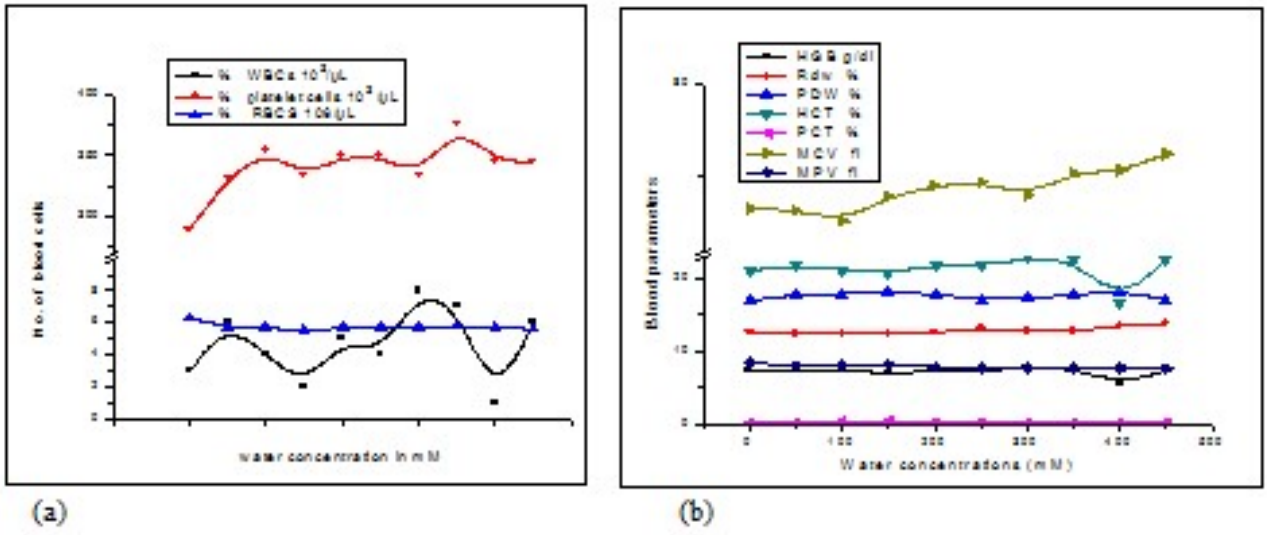

Fig. 6: (a) water concentrations versus blood cells. The figure shows bursting of the WBCs with higher water concentrations and (b) water concentrations versus blood parameters.

Table 1: Change in different blood parameters in the presence of sugar concentrations;

Sr. No.	Sugar Concentration (mM)	No. of WBCs (103/µL)	No. of platelet cells (103/µL)	No. of RBCs (106/µL)	HGB g/dL	RDW %	PDW %	MCV fL	MPV fL	HCT %	PCT %
1	0	6.3	289	2.88L	7.3L	12.6	17	73.3L	8.5	21.1L	0.25
2	50	5.9	284	2.88L	7.4L	12.4	17.4	73.3L	8.1	21.1L	0.23
3	100	5	253	2.9L	7.4L	13	17.9	73.4L	8	21.3L	0.2
4	150	4.4	259	2.86L	7.2L	12.8	17.2	74.1L	7.7	21.2L	0.2
5	200	4.6	244	2.85L	7.2L	12.9	17.3	74L	7.8	21.1L	0.19
6	250	4.4	256	2.81L	7.1L	13	17.3	73.7L	7.9	20.7L	0.2
7	300	4.3	240	2.86L	7.1L	12.6	18.3H	74.1L	7.6	21.2L	0.18
8	350	4.3	247	2.79L	7.1L	13.1	17.3	74.2L	7.6	20.7L	0.19
9	400	4.4	290	2.75L	7L	12.7	17.4	73.5L	8.2	20.2L	0.24
10	450	4.5	307	2.79L	7L	13.1	17.9	74.2L	8	20.7L	0.25
Sum		48.1	2669	28.37L	71.8L	128.2	175	737.8L	79.4	209.3L	2.13
Count		10	10	10	10	10	10	10	10	10	10
Average		4.81	266.9	2.837L	7.18L	12.82	17.5	73.78L	7.94	20.93L	0.213
Standard Deviation		0.72	23.43	0.05	0.15	0.24	0.40	0.38	0.28	0.34	0.03

Table 2: Change in different blood parameters in the presence of pure water concentrations.

Sr. No.	Water Concentration (mM)	No. of WBCs (103/µL)	No. of platelet cells (103/µL)	No. of RBCs (106/µL)	HGB g/dL	RDW %	PDW %	MCV fL	MPV fL	HCT %	PCT %
1	0	6.3	289	2.88L	7.3L	12.6	17	73.3L	8.5	21.1L	0.25
2	50	5.7	331	2.98L	7.6L	12.5	17.9	73.2L	8	21.8L	0.26
3	100	5.7	354	2.92L	7.5L	12.5	17.9	72.6L	8.2	21.2L	0.29
4	150	5.5	333	2.8L	7L	12.5	18.2	73.9L	8.3	20.7L	0.27
5	200	5.7	349	2.94L	7.4L	12.6	17.9	74.5L	7.9	21.9L	0.26
6	250	5.7	349	2.92L	7.4L	13.2	17.2	74.7L	7.7	21.8L	0.24
7	300	5.7	333	3.08L	7.8L	12.7	17.3	74L	7.7	22.8L	0.25
8	350	5.8	375	3.02L	7.8L	12.8	17.8	75.2L	7.8	22.7L	0.25
9	400	5.7	345	2.02L	5.5L	13.6	18.3	75.3L	7.7	16.7L	0.24
10	450	5.6	344	2.98L	7.5L	13.8	17.2	76.2L	7.6	22.7L	0.24
Sum		57.4	3402	28.54L	72.8L	128.8	176.7	742.9L	79.4	213.4L	2.55
Count		10	10	10	10	10	10	10	10	10	10
AVG.		5.74	340.2	2.854L	7.28L	12.88	17.67	74.29L	7.94	21.34L	0.255
Standard Deviation		0.212	22.12	0.302	0.668	0.482	0.46	1.10	0.30	1.78	0.02

toward elliptical shape and ultimately lyses. We observe that the complete disintegration of the WBCs as well as RBCs has been reached at 450mM. The size of the WBCs also changed, gradually increasing due to addition of extra glucose. Fig. 1 (a) shows normal size of WBCs ~13 µm. fig. 1(b) shows the trend of little increasing size of WBCs ad has been measured ~14 µm and fig. 1(c) shows rupturing of cells due to further swelling of cells (~29 µm). Overall size of cells increases (~24 µm) from top to bottom and inter cellular distance decreases (fig. 1(d)).

The shape of platelet cells changed as the concentration of sugar was increased. They were disc shaped and become ellongated after the addition of sugar as shown above in fig. 2(b). Then, suddenly they started to swell and prepared for lysis by increasing their size as shown above in fig. 2(c). The size of platelet cells then gradually started decreasing from 300 mM up to 450 mM as shown in fig. 2 (d).

Pure water's effect on blood components

We have analyzed two parameters of WBCs and platelets i.e. (i) shape of WBCs and (ii) size of WBCs before after admixing of distilled water. 0mM to 450mM of pure water causes changes in Shape of WBCs. fig. 4(a) represents the Normal shape of WBCs and become elliptical and finally lyses (fig. 4(b,c and d)). **f**ig. 4(a) shows normal size of WBCs (~13µm) and fig. 4 (b) shows the increase in the size ~ 20 µm). fig. 4(c) shows the much increase in the size ~ 25µm and 4(d) also shows swelling (~26µm) and bursting of cells. concentration of water (H_2O) up to 200 mM (fig. 5 (b-c)) shows the gradual incerase in the size of platelet cells. fig. 5 (d)sows the start up of bursting from 250 mM up to 450 mM. wheres fig. 5 (a) shows orignal shape is like a disc or plateas.

DISCUSSION

Complete blood count (CBC of each sample was performed using a celltac α hematological anlyzer. The measured data of the blood components and parameters is given in table 1 and is plotted in fig. 3(a,b). Hemoglobin (HGB) is a red pigment present in blood which plays a vital role in carrying oxygen to different parts of body. HGB levels gradually decrease with increasing concentration of glucose as shown in fig. 3(b). An abnormal value can be life-threatening. A low hematocrit (HCT) or erythrocyte volume fraction (EVF) value was noted with increasing concentration of sugar as shown in fig. 3(b). Low HCT can results in leukemia. Mean corpuscular volume (MCV) shows increasing trend under hyperglycemia as shown in fig. 3(b) and thus showing trend from normocytic anemia to macrocytic anemia. Red blood cells distribution width (RDW) is the measure of range of variations of RBCs. RDW shows an increasing trend with increasing concentration of sugar as shown in fig. 3(b). A high value of RDW also causes anemia mean platelet volume (MPV) is the measure of average size of platelet cells and is useful to predict the destruction or production of platelet cells (Chekol *et al*.). High MPV results destruction of platelet cells. Fig. 3(b) shows decreasing trend of MPV thus indicating production of cells. Platelet distribution width (PDW) is used to express the variations in size of platelet cells. PDW increases with increasing concentration of sugar. MPV and PDW are related with each other and are generally direct in relation but here is opposite condition as shown in fig. 3(b). Low MPV with high PDW result in anemia. Platelet crit (PCT) is a source to measure quantitative disorders/ abnormalities of platelet cells. PCT shows thrombocytopenia up to 350 mM and then thrombocytosis up to 450 mM as shown in fig. 3(b). Here, HGB, RDW, HCT and MCV are the parameters related to RBCs while MPV, PDW and PCT are related with platelet cells.

Now, we discuss in detail the changes with respect to cells count of leucocytes, thrombocytes and erythrocytes. Table 2 contains the variations in different blood parameters in the response of pure water concentrations. These parameters have been plotted in figure 6. In this work, we have investigated hypotonic solution. fig. 6(a) shows the decreasing trend in case of no of cells from 0 mM up to 450. HGB level starts increasing from 50 mM and remains high up to 350 mM and then starts decreasing as shown in fig. 6(b). So, it can be suggested that at very high concentration of pure water, HGB level in blood becomes low and cannot perform properly (Akyol *et al*.). Low HCT results anemia and leukemia. Its abnormal value is life threatening, a high HCT or EVF value is noticed against increasing concentration of water as shown in fig. 6(b). MCV shows increasing trend under hypernatremia as shown in fig. 4 and thus showing trend from normocytic anemia to macrocytic anemia. RDW shows decreasing trend up to 150 mM and with further increasing concentration of water it increases up to 450 mM as shown in fig. 6(b). Fig. 6(b) shows decreasing trend of MPV thus indicates production of cells. Thus it can be suggested that high usage of water is beneficial to overcome low platelet count in dengue patients. PDW increases with increasing concentration of water up to 400mM. MPV and PDW have been found in opposite condition here as shown in fig. 6(b).

CONCLUSION

We have analyzed the blood components and blood parameters under hyperglycemia and hypotonic condition. In comparison for table 1 and 2, we observe that blood components and blood parameters like WBCs, platelet cells , RBCs , HGB, RDW, PDW, MCV, MPV, HCT, and PCT, have slightly higher values in pure water containing blood samples as compared to sugar. The statistical analysis's predicted trend is given in table 3. We revealed

that shape deformation, cells lyses and changes in size are observed at higher concentration of sugar and water i.e. beyond 200 mM for sugar and 50 mM for pure water and complete burst approached at 450 mM. This concludes that extra usage of sugar and usage of distilled water is life threatening because many diseases are linked with diabetes like thrombocytopenia, thrombocytosis, anemia, leukemia, blood pressure and proliferative diseases etc. a significant variation has been observed in the size of the WBC i.e. 24 µm has been reached at 450 mM of concentrations. Diabetes disturbs these blood components and blood parameters and gradually affects their normal functioning.

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