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Original Article

Effect of direct electric current on human blood count

Jomana Mahmoud Diab[1*], Ahmad Souliman Ahmad[1], Abdulfattah Abbas[2], and Ward khalifa Abo-Amsha[3]

[1] *Faculty of Biomedical Engineering, Al Andalus University for Medical Sciences, Qadmus, Tartous, 0963-043 Syria*

[2] *Faculty of Medicine, Al Andalus University for Medical Sciences, Qadmus, Tartous, 0963-043 Syria*

[3] *Department of Biomedical Engineering, Faculty of Mechanical Engineering, Aleppo University, Aleppo, 0963-021 Syria*

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Abstract

Direct Current (DC) is widely used in electrotherapy for disease treatment, with typical studies on the effect of DC on disease factors in the blood. The objective of this work was to study the effects of DC on human blood, and to determine the intensity of DC that safely flows through blood, and to know which components of blood are most affected by DC. The effects on the counts or levels of main blood components [Red Blood Cells (RBCs), White Blood Cells (WBCs), Platelets (PLTs), Hemoglobin (Hb), and Hematocrit (Hem)] were considered. DC intensities from 1 to 10 mA were passed through blood samples of ten healthy persons for periods of up to one hour. A blood test (blood count with white blood cell differential) was carried out before DC flow and after 5, 10, 15, 30, 45, and 60 minutes of treatment. The study reveals that passing DC through human blood has no effect on blood count when the intensity is less than 3 mA, and it is safe to flow a DC of 3 mA for a period of one hour. The smallest effect of DC flow is on RBCs, Hb, and PLTs, whereas the greater effects are on Hem and WBCs. DC flow affects all types of WBCs in the same way.

Keywords: blood count test, white blood cell differential test, blood cells, percentage decrease of count, safe direct current

1. Introduction

Blood has many essential functions in the body. It transports oxygen, nutrients, and constitutional elements to tissues and returns carbon dioxide, ammonia, and other waste products from these tissues. In addition, it has a vital role in the immune system, and in regulating body temperature. Blood consists of two main components: plasma, which makes up 45-55% of blood volume, and formed cellular elements, which include red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs). These formed elements combine together to make up the remaining blood volume. Plasma is made up for 90% by water, 7-8% soluble proteins, 1% electrolytes, and 1% elements in transit (Robinson *et al*., 2007).

RBCs, also known as erythrocytes, have roughly the shape of a biconcave disk. This shape allows the RBCs to carry oxygen and pass through even the smallest capillaries in the lungs. RBCs contain hemoglobin (Hb), which transports oxygen from the lungs to the rest of the body. The hematocrit (Hem) test measures the volume percentage of RBCs found in blood. RBCs live about 120 days and do not self-repair (Robinson *et al*., 2007).

The different types of WBCs, also known as leukocytes, include basophils (BA), eosinophils (EO), neutrophils (NE), monocytes (MO), and lymphocytes (LY). The BA store

*Corresponding author
Email address: j.diab@au.edu.sy;
jomanad162@gmail.com

and synthesize histamine, which is important in allergic reactions. The EO fight parasites and contribute to allergic reactions. Normally, there are only a few EO found in the peripheral circulatory system. The NE are the first to act when there is an infection and, also, the most abundant WBCs. The NE fight bacteria and viruses by phagocytosis. The MO are the largest size WBCs and are responsible for rallying the cells to defend the body. The LY help our immune response (Robinson *et al*., 2007).

PLTs, also known as thrombocytes, are membrane-bound cell fragments. The sticky surfaces of the platelets allow them to accumulate at the site of an injured blood vessel to form a clot. This aids in the process of hemostasis. The circulating life of a platelet is from eight to ten days (Robinson *et al*., 2007).

A blood test is a laboratory analysis performed on a blood sample that is usually extracted from a vein in the arm, using a needle, or via a finger prick. The most common blood test is the complete blood count (CBC). WBCs count, a part of CBC, gives information on the presence of a disease or condition affecting the body, but it cannot determine the underlying cause. Therefore, other tests, such as WBCs differential, may be required. The blood differential test, or WBCs differential count, measures the percentage of each type of WBCs in blood. The normal adult has a physiological normal range of CBC and a normal percentage of an overall WBCs count (WBCs differential), Lokwani, D. P. (2013).

Blood has electrical properties due to the presence of electrolytes in the plasma and the presence of large organic and electrically-polarized molecules, and plasma conductivity depends on the electrolyte content and concentrations (Al-Badri, 2015). Some of these electrolytes include salts, colloidal electrolytes, and particularly proteins (Hirsch *et al*., 1950). In addition, most blood components are negatively charged since PLTs, RBCs, WBCs, and proteins are attracted to the point of injury, which usually has a positive charge (Hayashi, 1968). RBCs surfaces are negatively charged due to the presence of the carboxyl group of sialic acids in the red blood cell membrane (Fernandes, Cesar, & Barjas-castro, 2011).

Many researchers have studied the effects of magnetic fields on blood. For example, the effect of high static magnetic fields on platelets (Higashi *et al*., 1993), on diamagnetic orientation of blood cells (Yamagashi *et al*., 1992), and on viscosity of blood (Tao & Huang, 2011; Yousef, Vinay, & Ching-Jen, 2001). In addition, the effects of alternating magnetic fields on RBC properties (Ali, Mohamed, & Mohamed, 2003), and their therapeutic effects (Bhatti, Zeeshan, & Ellahi, 2017; Fergany *et al*., 2017) were investigated. Hye and his team investigated whether exposure to electromagnetic field from 915 MHZ radiofrequency identification system affected circulating blood cells in rats (Hye *et al*., 2017).

The effects of alternating currents (AC) on blood were also investigated, such as their effects on the transport of red blood cells in micro capillaries (Minerick, Ostafina, & Chang, 2002) and on the electrical properties of blood (Schwan, 1983). AC is usually used to measure dielectric properties of blood (Al-Badri, 2015; Minerick, Ostafina, & Chang, 2002), including dielectric parameters such as dielectric constant, as well as conductivity (Altaf & Ahmad, 2017; Hirsch *et al*., 1950).

Blood electro-coagulation produced by the direct current (DC) was investigated early on. Hayashi (1968) reviewed all related studies between 1824 and 1964 (Hayashi, 1968). According to Hayashi, Schwatz in 1959 found that a DC of 4.5 V and 5 mA that is applied for an hour was able to cause thrombi in a dog's superficial femoral vein. Hayashi also reports that Kravitze observed, in 1964, that a DC of 12 to 16 mA used for a period of 7 to 10 minutes was usually sufficient to produce coagulation on a diffuse bleeding surface. Haruo Hayashi, himself, observed microscopically that the process of intra-vascular thrombus formulation started when a 6 V, 4 to 4.5 mA positive DC was applied for a period of 9 to 12 minutes upon the mesenterial pilary of a rabbit.

Therapeutic effects of DC were discussed, such as the effect of low-level DC on Aides (Lyman *et al*., 1991), carcinoma (Griffin *et al*., 1994, 1995), blood vessels (Maor *et al*., 2007), and on ablation of neoplastic (Taylor *et al*., 1994).

Transcranial direct current stimulation (tDCS) is a theraputic technique in neuropsychiatry, which has been used to treat a wide range of neurological and psychiatric disorders. DC is delivered continuously at 2 mA for 20 minutes using cathode and anode positioned over cranium (Veronica, Angelo, Donel, & Colleen, 2013). DC applied in tDCS increases cerebral blood flow (Zheng, Alsop, & Schlaug, 2011).

Electrical stimulation helps in wound healing because it attracts neutrophils and macrophages, and increases blood flow (Gentzkow, 1991). DC stimulation has been used, experimentally, to treat a wide range of wounds such as thoracic spine wound (Fitzgerald, & Newsome, 1993), healing of wounds and pressure sores (Bogie, Reger, Levine, & Sahgal, 2000), and pressure ulcers in pigs (Kambic, Reyes, Manning, Waters, & Reger, 1993; Reger, Hyodo, Negami, Kambic, & Sahgal, 1999). Recently High-voltage Pulsed current (HVPC) is used to treat patients with diabetic foot, venous leg, and pressure ulcers. HVPC requires the application of very high peak-current amplitude (2-2.5A) (Polak, Franek, & Taradaj, 2014).

There is a lack of investigations regarding the effects of DC flow through human blood on its components. Haruo Hayashi (1968) in his research on rabbit observed that blood pressure, PLTs and Hem (factors involved in clotting mechanism) changed when DC (10, to 37mA) is applied for a periods of 5 to 25 minutes. He concluded only that "PLTs were decreased and Hem was increased" (Hayashi, 1968). Lyman *et al.* passed a 1 to 100 microampere DC for a period of up to 12 minutes through blood infected with HIV. They only studied the effects of DC on HIV virus and reported that DC inactivated the virus and stopped its replication (Lyman *et al*., 1991).

Direct Current (DC) is widely used in electrotherapy for disease treatment. Researches usually study the effects of DC on a disease-causing factor found in blood (e.g. HIV virus), on carcinoma, on wound healing, on cerebral blood flow, on brain disease, and on blood vessels. There is a lack of investigations regarding the effects of DC flow through human blood on its components.

Although passing DC through blood helps in healing diseases and wounds, the question is: are there any harmful effects on the components of blood? Furthermore, what are the limits of safe current intensity and duration of current passage?

Acknowledging the possible negative effects of DC used in therapy, in electro-surgery, in blood-testing laboratory devices, and in blood apheresis is an important matter and must be taken into consideration. Knowing the safe limits of intensity and duration of DC flowing through blood ensures that these

limits are maintained when using DC in medical applications.

Because the effects of DC flow on blood components have not been considered in previous researches and the component counts of human blood have never been investigated before, we investigated the effects of low-level DC (1 to 10 mA) on the main blood component counts (WBCs, RBCs, Hb, Hem, and PLTs), when applied for a period of 1 to 60 minutes. We determined the intensity of DC that safely flows through blood, the components of blood that are most affected by DC, and the safe minimal value of blood count.

2. Materials and Methods

2.1 Electric circuit

The DC electric circuit used in this study (Figure 1) consisted of an AC-DC adapter PI-41-77V-3 (Braun, Kronberg, Germany), a potentiometer RV16AF10-15R1-B20K (Alpha, Taiwan), and an electrical switch 97510 International configuration (Enfield, Ct.). An electric current meter DT-9205A (Shenzhen, China), was used to measure the DC intensity. To ensure that the current passes through the entire bloodstream, the ends of the electrical wire that enter the blood tube were coiled in a spiral forming four concentric circles, the largest of which has an 8-mm diameter. The blood tube used was made of plastic and has a length of 6 + 1 cm and a diameter of 1.4 cm and 0.8 cm, and has a hole used to fill and draw blood (Figure 2). Blood cell count was measured using a laboratory CBC device MEK-6510 (Nihon Kohden, Tokyo, Japan).

2.2 Laboratory test

Venous blood samples were drawn from ten healthy adult (volunteer) donors (six women, four men) in a blood center. The blood was collected into anticoagulant Ethylene Diamine Tetra-acetic Acid (EDTA) tubes Vacumed® (FL medical, Itally, padova), and kept in a refrigerator at 4 °C until use. All investigations have been made within one to two hours after collection. All experiments have been performed at room temperature.

The potentiometer was first set at a value proportional to the desired current. Next, 5 cm^3 of blood were put into the blood tube. The switch was then turned off and the desired current intensity was adjusted by slightly tuning the potentiometer and monitoring the current meter. The meter was then removed and the switch was turned on. At the desired time, a blood sample was drawn from the tube to perform the CBC test. Since DC causes blood cells to agglomerate, before drawing the sample the current flow is stopped, the tube is shaken to mix blood components, and the current flow is allowed again.

A part of CBC count, including WBCs (quantity of WBCs, thousand/mm^3), RBCs (quantity of RBCs, million/mm^3), Hb (amount of hemoglobin, g/dL), Hem (percentage of RBCs in the blood, %), and number of PLTs (thousand/mm^3) is performed using the CBC device.

Blood differential test is also performed using the CBC device, WBCs differential count involves the following five cell types: neutrophils (NE, percentage of NE in the blood, %), lymphocytes (LY, percentage of LY in the blood, %), monocytes (MO, percentage of MO in the blood, %), eosinophils (EO, percentage of EO in the blood, %), and basophils (BA, percentage of BA in the blood, %).

2.3 Statistical analysis

SPSS ver. 19.00 for Windows was used for statistical analysis (IBM, US). The different effects of DC on blood components were studied using Analysis of Variance (ANOVA) test followed by Fisher's Least Significant Difference (LSD) test. The LSD test determines the most affected component. The relationships between DC intensity and time of flow as independent variables, and ($\overline{\Delta\%}$), as a dependent one, were analyzed using the Multivariate Regression test. A P-value <0.05 was considered statistically significant.

3. Results and Discussion

In this study the effects of low-level DC intensities (≤ 10 mA) on blood count of ten healthy adults have been investigated. DC intensities of 1 to 10 mA have been allowed to flow through the blood tube for periods up to an hour (Figure 1). A blood test (CBC with WBCs differential) has been performed before and during the flow of DC through blood stream for intervals of 5, 10, 15, 30, 45, and 60 minutes.

The percentage decrease of the count for each component of blood is calculated for all DC intensities at all considered intervals. Δ% is calculated as follows:

$$\Delta\,\% = \frac{b - a}{b} \times 100 \qquad (1)$$

where b and a are the blood count before and after the flow of DC respectively. The mean value ($\overline{\Delta\%}$) and standard deviation (SD) of Δ% (of ten values) are calculated at each DC intensity and time of flow (Table 1).

The safe minimal value of blood count (SMV) when a DC intensity of 10 mA is applied for a period of 60 minutes

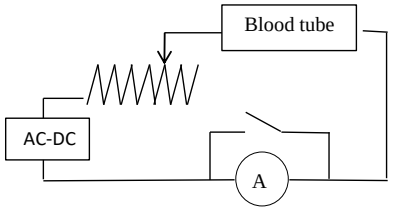

Figure 1. Schematic diagram of the DC circuit. It consists of an AC-DC adapter PI-41-77V-3, a potentiometer RV16AF10-15R1-B20K, an electrical switch 97510 International configuration, an electric current meter DT-9205A, and a blood tube

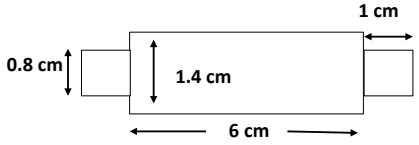

Figure 2. A schematic diagram of the blood tube used in the DC circuit

Table 1. The percentage decreases of the counts ($\overline{\Delta\%}$±SD) of CBC (WBCs, RBCs, Hb, Hem%, and PLTs), when DC of 3mA or 10mA flows for a period of 10, 15, or 60 minutes.

Direct current		WBCs thousand/mm3	RBCs million/mm3	Hb g/dl	Hem %	PLTs thousand/mm3
Intensity [mA]	time [min]			$\overline{\Delta\%}$ ± SD		
3	10	0.6 ± 0.45	0.41 ± 0.31	0.69 ± 0.43	0.54 ± 0.83	0.15 ± 0.19
10	15	8.1 ± 0.59	6.36 ± 0.24	2.93 ± 0.43	8.48 ± 0.63	5.31 ± 0.26
10	60	32.26 ± 0.69	12.39 ± 0.28	14.35 ± 0.48	46.14 ± 0.82	12.91 ± 0.32

RBCs (Red blood cells), WBCs (white blood cells), PLTs (platelets), Hemoglobin (Hb), and Hematocrit (Hem)

$\boldsymbol{\Delta}\ \% = \frac{\mathbf{b-a}}{\mathbf{b}} \times 100$, where b and a are the blood count before and after the flow of the current respectively.

(referred to as 10/60) (Table 2) is calculated using the proposed formula:

$$\text{SMV} = \frac{100 \times \text{m}}{100 - \overline{\Delta\%}\,|_{10/60}} \qquad (2)$$

where m is the minimum value of the normal range related to the blood component's count (we consider minimum values of women) (Table 2) and $\overline{\Delta\%}|_{10/60}$ is $\overline{\Delta\%}$ value at 10/60.

A statistical analysis is carried out using ($\overline{\Delta\%}$) of each blood component at different values of DC intensity (3 to 10 mA) and time of flow (5, 10, 15, 30, 45, and 60 minutes). The data set consists of forty eight (48) data points for each blood component, each point has three elements ($\overline{\Delta\%}$, DC intensity, time of flow). Firstly, the effect of DC on each component of blood is studied using the ANOVA and LSD tests. Next the relationships between $\overline{\Delta\%}$, as a dependent variable, and the time of flow and DC intensity, as independent variables, are analyzed using the Multivariate Regression test (Table 3).

To compute the values of DC intensities that safely flow through human blood, firstly, the safe values of Δ% ($\Delta\%_{\text{Safe}}$) are computed, which relates to the decrease of the count from the b value to the minimum value of the normal range, then the mean value of $\Delta\%_{\text{Safe}}$ ($\overline{\Delta\%_{Safe}}$) is computed. The safe value of DC intensity is the value that corresponds to ($\overline{\Delta\%}$) less than $\overline{\Delta\%_{Safe}}$ (Table 4).

The experimental results indicate that DC intensity (greater than 2mA) flow through blood decreases the count of the essential blood components (WBCs, RBCs, Hb, Hem, and PLTs). The amount of decrease is related to DC intensity and duration of flow ($p<0.0001$) (Table 3). However, the percentage of each type of WBCs is not affected, which indicates that DC flow affects all types of WBCs in the same way. Intensities of 1 mA and 2 mA flowing for a period of 60 minutes do not have any effect on blood since the count of any component did not change at all. DC of 3/10 (3 mA flows for a period of 10 minutes), has negligible $\overline{\Delta\%}$ (< 0.7%). DC of 10/15 affects blood slightly ($\overline{\Delta\%}$ < 9%) for all blood components (Table 1). DC flow of 10/60 affects RBCs (Δ% = 12.39 ± 0.28), PLTs (Δ% = 12.91 ± 0.32), and Hb (Δ% = 14.35 ± 0.48), slightly, but affects WBCs (Δ% = 32.26 ± 0.69), and hem (Δ% = 46.14 ± 0.82) considerably (Table 1).

From a statistical point of view, the effects of DC are not similar on all blood components (ANOVA, $P < 0.001$). The largest effect of DC is on Hem, then on WBCs (the LSD test is statistically significant). The counts of these two components decreased significantly when current intensity was increased. However, the effects of DC on the counts of RBCs, PLTs, and Hb are insignificant (Figure 3 and Figure 4).

Linear multivariate regression statistical analysis showed that there is a relationship ($p < 0.0001$) between DC intensity and time of flow, as independent variables, and ($\overline{\Delta\%}$), as a dependent one (Table 3). The resultant linear equations can be useful for estimating Δ% related to any DC intensity for any period of time.

DC of 10/60 can flow safely only for people who have counts equal to or greater than SMV values (Table 2). It can be noted, that all SMVs of blood count reasonable since they are less than the maximum value of the normal range related to the blood component's count (Table 2). However, the case of Hem is not: regarding Hem, a DC of 10 /15 can be safe since SMV is 38.68% (this value is reasonable).

The safe DC is the current that keeps the count greater than minimum value of the normal range. We found that 3/60 is safe for all blood components (Table 4). DC up to 10/60 is safe for RBCs, PLTs, and WBCs. However, for Hb a DC up to 6/60 is safe, but a DC of 7 to 9 mA is safe only for a period of 45 minutes. Regarding Hem, safe DC is up to 10/15 (Table 4).

Thrombus starts to form when a DC intensity of 10 mA is applied for a period of about 45 minutes. This DC intensity is large compared to the value (4.5 mA) reported in (Hayashi, 1968). The reason for this can be attributed to the presence of the anticoagulant EDTA in this research.

DC flow through blood slightly affects RBCs, but it significantly affects Hem. One reason for this could be that DC flow may cause the size of RBCs to shrink, thus, their volume percentage with respect to total blood volume decreases. Other reasons, which need to be studied, may also contribute to this outcome.

The results of this research can be useful when using DC to treat diseases such as cancer, AIDS, or to induce wound healing. In addition, the results can be useful when dealing with electric devices that handle human blood (e.g. apheresis machines and other blood-testing laboratory devices). The possibility that a DC current passing through the tested blood influences it must be taken into consideration, and test results may need to be adjusted accordingly.

Table 2. Safe minimal value (SMV) of blood count when DC flows at 10/60 (current intensity of 10 mA and time period of 60 minutes)

Blood Component	SMV	Normal Range
WBCs	6.64 [thousand/mm^3]	4.5-11 [thousand/mm^3]
RBCs	4.79 [million/mm3]	4.6-6 [million/mm3] for men, 4.2-5 [million/mm3] for women
Hb	14.01 [g/dL]	13.3-16.2 [g/dL] for men, 12.0-15.8 [g/dL] for women
Hem	65.72%	38.8-46.4 % for men, 35.4-44.4 % for women
PLTs	172.23[thousand/mm3]	150 to 450 [thousand/mm3]

RBCs (Red blood cells), WBCs (white blood cells), PLTs (platelets), Hemoglobin (Hb), and Hematocrit (Hem)

Table 3. The results of the Multivariate Regression for WBCs, RBCs, Hb, Hem, and PLTs. The percentage decrease of the count ($\overline{\boldsymbol{\Delta}\%}$) is the dependent variable and the DC intensity and duration of flow are the independent variables.

Blood component	Linear equations	P-value
WBCs	Δ%= 0.34t+1.61I-8.96	<0.0001
RBCs	Δ%= 0.062t+0.71I-3.71	<0.0001
Hb	Δ%= 0.15t+0.59I-4.04	<0.0001
Hem	Δ%= 0.673t+1.92I-15.773	<0.0001
PLTs	Δ%= 0.13t+0.75I-4.05	<0.0001

RBCs (Red blood cells), WBCs (white blood cells), PLTs (platelets), Hemoglobin (Hb), and Hematocrit (Hem)
$\boldsymbol{\Delta}\,\% = \frac{\mathbf{b-a}}{\mathbf{b}} \times \mathbf{100}$, where b and a are the blood count before and after the flow of the current respectively.

Table 4. Safe intensities of DC and corresponding times of flow for WBCs, RBCs, Hb, Hem, and PLT

WBCs		RBCs		PLTs		Hb		Hem	
DC [mA]	Time [min]	DC [mA]	Time [min]	DC [mA]	Time [min]	DC [mA]	Time [min]	DC [mA]	Time [min]
3 to 10	60	3 to 10	60	3 to 10	60	3 to 6 7 to 9	60 45	3 to 10 3	15 60

RBCs (red blood cells), WBCs (white blood cells), PLTs (platelets), Hemoglobin (Hb), and Hematocrit (Hem)

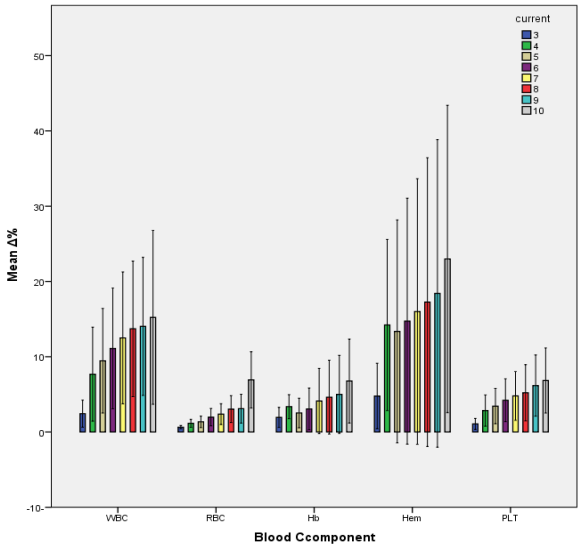

Figure 3. The mean percentage decreases of each count [$\overline{\Delta\%}$] of blood components (WBCs, RBCs, Hb, Hem, and PLTs) at current intensities of 3 to 10 mA. DC current has a significant effect on the counts of Hem and WBCs, but smaller effects on the counts of RBCs, PLTs, and Hb

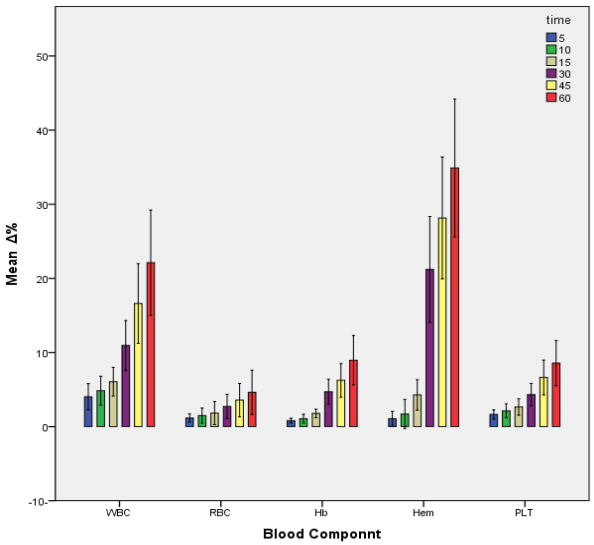

Figure 4. The mean percentage decreases of counts **[**$\overline{\Delta\%}$**]** of blood components **[**WBCs, RBCs Hb, Hem, and PLTs**]** for various durations of DC flow [time (min)]. Time duration of DC flow has significant effects on the counts of Hem and WBCs, and less effects the counts of RBCs, PLTs, and Hb

4. Conclusions

It can be concluded from the previous discussion that DC intensities of 1 and 2 mA do not affect blood counts. DC of 3 mA can safely flow for a period of one hour. DC flow affects all types of WBCs in the same way. Statistical analysis indicates that the effects of DC are insignificant with regard to RBCs, PLTs, and Hb, in contrast to Hem and WBCs, on which the effects can be considerable.

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