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"Modulation of Whole Blood Transmittance by Static Magnetic Fields: An In-Vivo Analysis"

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Abstract: In this work, the transmission behavior of whole blood was investigated in-vivo experiments using a spectrophotometer. Female albino rats were exposed to 2.4 mT of static magnetic field for four selective weeks (1 hr, 2 hrs, 4 hrs, 6 hrs, and 8 hrs per day). One hour of exposure for 1 week increased the transmittance percentage up to 50% and shifted the peak to about 20 nm. At the 4 hours of exposure per day, only four weeks of exposed animals shifted the transmission curve upward. A similar variation was observed due to the 6 hrs and 8 hours/day exposure during 3 weeks. Therefore, other exposure periods declined the transmittance line below the control value. The 8 hrs/day post-exposure changed the curve line comparable to the exposed curve line, except for the 3 weeks exposure in which both exposure and post-exposure curves are aligned up with the same scale. We believe that the reason for shifting transmission curves is due to a change in the distribution pattern of the main blood cells as they respond to the magnetic field.

Keywords: *Static Magnetic Field; Spectrophotometer; Transmittance*

1. Introduction

Static magnetic field (SMF) is a zero-frequency field produced via either passing a direct current (DC) through a coil or from a permanent magnet [1]. Humans are targeted with SMF from various sources, including DC transmission lines, battery-operated motors, audio speaker components, and microwave ovens. These artificial sources can transmit SMF as small as 0.02 mT to 10 mT at 1cm away from the sources [2]. The intensity of the magnetic field (MF) decreases with the square distance from the emission point [3]. Studies showed the difficulty of shielding SMF as it can easily penetrate the body tissues freely [4]. Magnetic field can interact with mobile charges such as the ions, protons, and the magnetic materials of tissues, such as the red blood cells' magnetic moment [5].

Blood is a specialized connective tissue consisting of formed elements (erythrocytes, leukocytes, and platelets) and a fluid component called plasma. Important functions of blood are transportation, such as transporting oxygen (O2), carbon dioxide (CO2), nutrients, proteins, waste products, ions, hormones, and formed elements, regulation, and protection. Red blood cells (RBCs) are one of the essential formed elements of blood, non-nucleated at the mature stage and lacking cell organelles. A special protein pigment invaded a 90% of RBC weight called hemoglobin (Hb). The red colour of the cell is due to the presence of Hb content. Each of the RBCs is a biconcave disk-like shape of a 2 µm thick and has an average diameter of about 7.5µm, a surface area of 120–140µm^{2,} and a volume of 78–86 µm^3. They last in blood circulation for 120 days [6], [7]. Blood parameters can act as paramagnetic or diamagnetic. However, RBCs act in two different ways: paramagnetic or diamagnetic, depending

on their oxygenated state [8]. Hemoglobin is a molecule that carries oxygen and carbon dioxide. Each Hb has four binding sites attached to iron molecules. Haemoglobin iron molecules can be another reason why RBCs can behave as paramagnetic [9].

Due to the fact that most of the SMF from man-made sources and transportation systems emit about 2 mT, this study aims to investigate the influence of a 2.4 mT SMF on the whole blood optical transmission properties. A setup of an SMF was designed that emits a 2.4 mT uniform static magnetic field. According to WHO (1998), the transmitted magnetic field close to electric generating stations exceeds 2 mT. A similar SMF intensity was found 1m above the surface inside trams, trains, and hybrid cars [10], [11]. A 2 mT (50 and 60 Hz) exposure for 20 min increased the practice-related spatial memory in rats [12]. A 3 mT (60 Hz) MF effect on human cognitive performance showed an induce of abolition at improvement associated with practice [13]. A 3 mT (50 Hz) exposure during 50 days caused oxidative stress via increasing MDA levels in male Wistar rats [14]. An in-vivo exposure with 1.4 mT PMF during 30 days decreased blood parameters [2]. An in-vitro experiment illustrated that 1 mT and 6 mT (50 Hz) affected the PLT aggregation [15]. The effects of 2.49 mT (50 Hz) on HeLa cancer cells proliferation in Chinese Hamster Ovary (CHO) were examined by [16]. Cellular proliferation rate increased by 66.6%. An in-vivo exposure of (3-10) mT attributed suppression of blood pressure in male rats after a 7-week period [17]. A contemporary investigation has elucidated the impact of magnetic resonance imaging (MRI) on hematological components and biochemical indices. The findings of the investigation assert a significant elevation in the levels of hepatic enzymes (GPT, GOT, ALP) and thyroid-stimulating hormone (TSH), as well as the presence of liver-thyroid dysfunction in elderly females afflicted with hepatic disorders [18].

Previously, the influences of 2.4 mT SMF on blood parameters, such as erythrocyte counts and morphology, blood flow and viscosity, WBCs and PLTs counts were shown in our laboratories and research center. Therefore, the effect of this range of SMF on the optical properties of blood has not been shown yet. We believe that the effect of 2.4 mT of blood optical property requires an investigation. This study demonstrates the influence of SMF on whole blood transmittance properties using a UV-2900 spectrophotometer device.

2. Materials and methods

2.1 Animal participation

Female albino Rats participated in the study, each weighing approximately 190±10 grams. The experimental design involved dividing a total of 100 rats into five primary groups, with each group comprising 20 individual specimens. Subsequently, these primary groups were further subdivided into five distinct subgroups (designated as A, B, C, D, and E), with each subgroup consisting of four rats housed within a standardized plastic enclosure. Subgroup A served as the control group within each primary group. The remaining subgroups, B, C, D, and E, were subjected to exposure to a Static Magnetic Field for durations of one, two, three, and four weeks, respectively. Each of the primary groups numbered 1 through 5 experienced a predetermined duration of exposure per day, specifically 1, 2, 4, 6, and 8 hours per day, respectively. The rats were sourced from the Biology Department of the College of Education at Salahaddin University and were maintained in an animal facility that adheres to the ethical standards established by the university's committee for experimental animals, receiving a standardized diet and water, with a photoperiod of 12 hours of light and 12 hours of darkness at a controlled temperature of 22±2 °C.

2.2 Exposure setup

A highly homogenous static magnetic field was established through the utilization of six Helmholtz coils interconnected in a series configuration, as illustrated in Figure 1. The coils were evenly spaced

and meticulously calibrated. The generated magnetic field within the apparatus was quantified using two Teslameters, a Hall probe, and a digital Teslameter. An applied direct current facilitated the generation of a magnetic field strength of 2.4±0.2 mT within the plastic cage (at an angle of 360 degrees). Wooden bases were employed to securely position the plastic cages between the coils during the exposure period. The background magnetic field was disregarded, and the experimental setup was devoid of any shielding mechanisms. All experimental groups underwent exposure seven days per week, from 08:00 to 16:00.

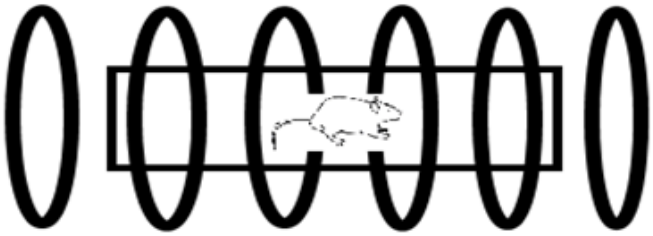

Figure 1 illustrates the arrangement of coils. A total of six identical coils are systematically aligned to produce a Static Magnetic Field (SMF), meticulously engineered within our research facility. Each coil possesses a diameter of 20 centimeters, and they are uniformly distributed in their configuration.

2.3 Blood collection

The albino Rats were anesthetized with a 0.4 ml of xylazine and ketamine hydrochloride mixture. The Blood specimens were withdrawn from the cardiac puncture into tubes, 5 mL inner wall-covered EDTA.

2.4 Blood sample examination

One milliliter of collected blood was loaded into the spectrophotometer and hit with the photon. Transmittance was measured under a limited wavelength range of 400-1000 nm.

Figure 2: UV-9200 Spectrophotometer

3. Results and discussions

Figure 3 shows the transmittance behavior of whole blood under the influence of SMF due to 1 hr and 2 hours of exposure per day. Figure 3A illustrates the increase in transmittance after 1-4 weeks of exposure for 1hr per day. Two and three weeks of exposure enhanced the transmission by about 30% and 40%, respectively. The one week and 4 weeks of exposure raised this behavior to a 50% increase. The peak of the transmittance shifted steadily from 640 nm to 660 nm. Comparable to that, the 2 hrs exposure per day (figure 3B) raised the transmittance percentage at 2 weeks and 4 weeks exposure only and by 35%-45%. The other exposure period lay as flat as that observed in the control samples. Therefore, the peak of the 4-week exposure has shifted significantly from 640-880 nm.

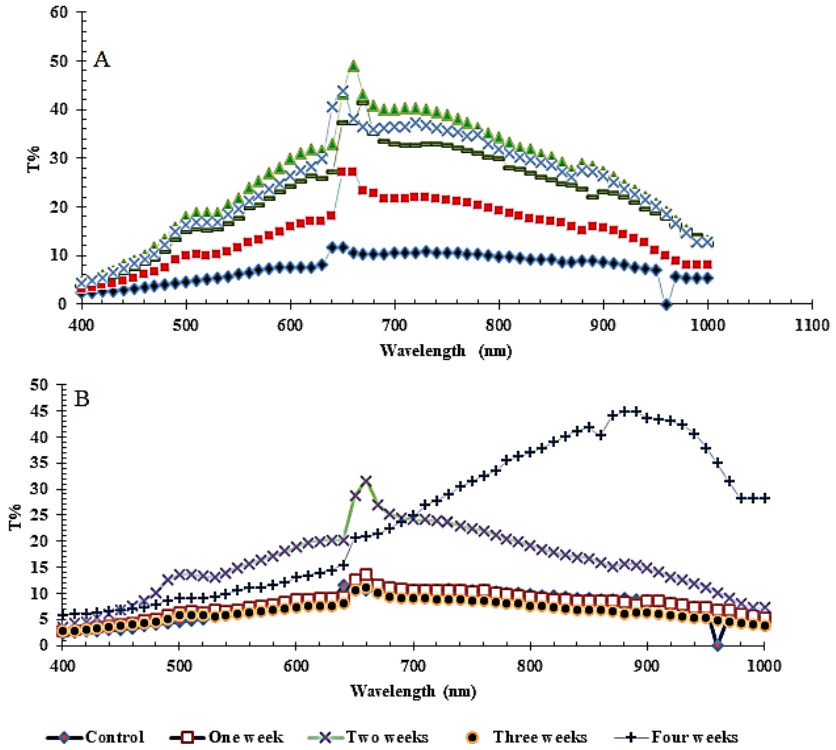

Figure 3: Effects of SMF on whole blood transmittance under 1 hr (A) and 2 hrs (B) exposure per day during several weeks. T: transmittance

Figures 4 and 5 represent the transmittance of 4 hrs, 6 hrs, and 8 hrs exposure for 1- 4 weeks. Four hours of exposure (Figure 4A) enhanced the transmittance percentage continuously, while the other exposure weeks remained as flat as the control values. On the other hand, 6 hrs exposures (figure 4B) demonstrated decline shifts and incline shifts due to one to two weeks and three to four weeks, respectively. The peak shift is only about 20 nm. The 6 hrs and 8 hrs (figure 5) observations are closely relevant, in which the 3 weeks exposure raised T% only slightly. The other exposure weeks remained below the level of the control transmittance line. The peak has a shifter with ±20 nm compared to the control line.

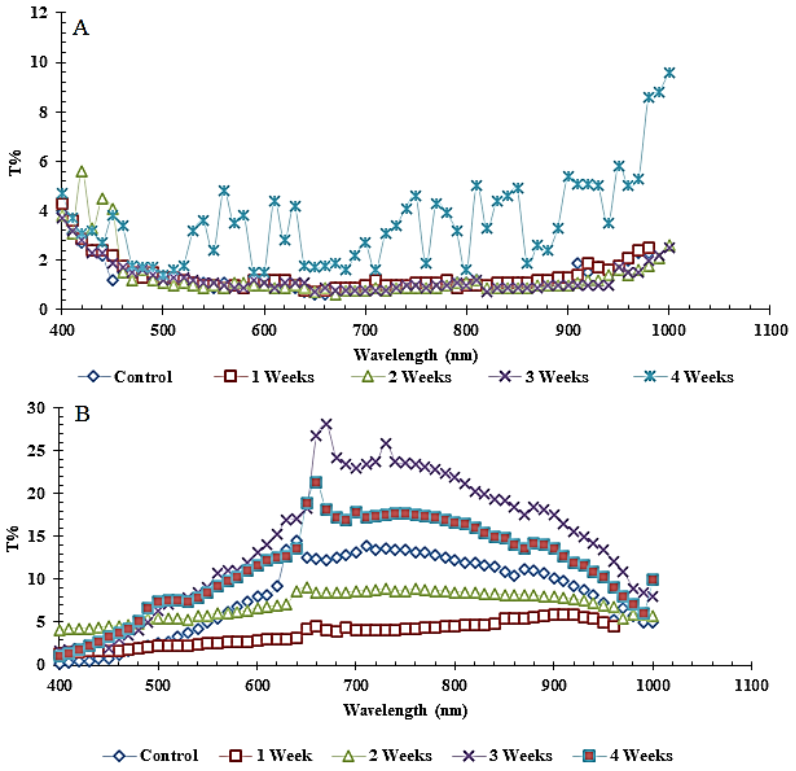

Figure 4: Effects of SMF on whole blood transmittance under 4 hr (A) and 6 hr (B) exposure per day during several weeks. T: transmittance

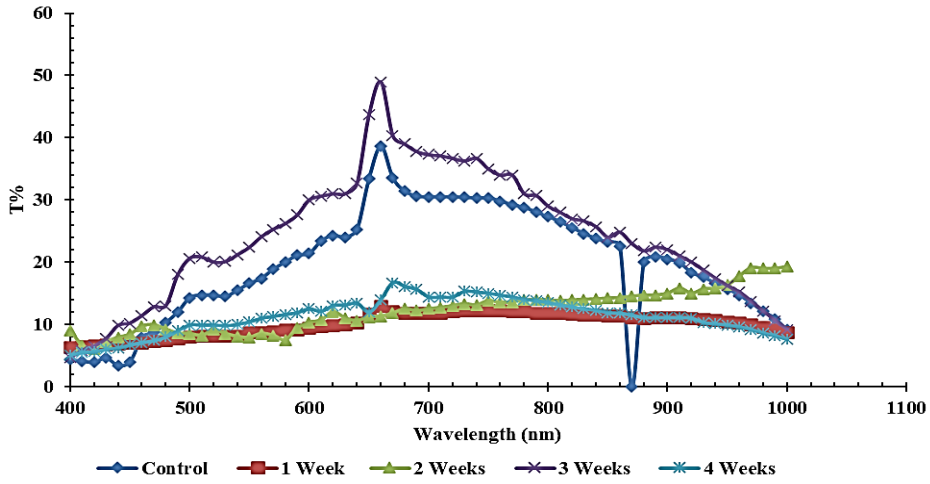

Figure 5: Effects of SMF on whole blood transmittance under 8 hr exposure per day during several weeks.

The optical properties increased where fewer megamolecules were available in the medium. In blood, the megamolecules are the RBCs that can absorb light efficiently. Since there is an aggregation of the blood RBCs, most of the cells are accumulating in limited spaces, subsequently, more space is available for light to transmit, causing an increase in the transmittance of light under specific SMF exposure. This figure is applied to all properties shown in Figure 3-5.

To examine the effects of post-exposure, transmittance was measured for several samples. Figures 6 and 7 represent the property at post-exposure for various periods. The 4 hr and 8 hr post-exposure effects are illustrated in Figure 6 (A and B), respectively. Four hours of exposure did not vary the transmittance percentage 7 days after exposure. However, 8 hours/day exposure has further declined

transmittance at 24 hrs post-exposure. 24 hours post-exposure was shown for 1-4 weeks after 6 hr exposure/day. One week post-exposure curve has shifted back above the control line. Three and four weeks post-exposure remained the same as exposed samples after 24 hrs of exposure. Therefore, two weeks post-exposure shifted the transmission peak above 900 nm.

The overall exposure effect is as follows: a short exposure time per day (1 hr/day) and a long exposure time per day (6 hrs/day) increased the blood transmittance. That may have occurred due to blood aggregation. Aggregations can cause the transmission compared to uniformly spreading blood RBCs. This figure resulted in less allowance for blood transmittance at 8 hours/day exposure. Other exposure periods did not affect the optical properties of blood significantly. Regarding the post-exposure, all blood properties return to similar levels as the control properties of blood samples after 1 week of exposure.

Blood properties variation can alter the physical properties of whole blood. RBCs' aggregation led to an increase in blood viscosity due to clusters that form in different sizes and shapes. This aggregation is macromolecular and may also happen among RBCs and PLTs. Each of these large and small molecules in blood possesses optical properties, including absorbance and transmittance. Consequently, the viscosity variation can affect the transmittance properties of blood. Blood cell aggregations were shown intensively. Additionally, blood cells also respond non-linearly to applied SMF. Other property variations can be cell structure, such as membrane and Hb content. Mustafa et al [19] showed that the 42.5 mT SMF increased blood cells after 2 min and 30 min exposure in-vitro experiment. Haemoglobin (Hb) was affected significantly after 2 min of exposure, and Hct% at 30 min of exposure. RBC-PLT and RBC-RBC aggregation were found under a certain exposure period [19]. In another study, WBCs and PLT counts varied significantly after 2.4 mT exposure in-vitro. For instance, WBC counts increased by 32% and PLTs declined by 29% after 15 min of exposure. The study believes that the increase in blood cell counts can cause blood viscosity to reduce [20]. In an in-vivo study, PLT counts declined by 51% after 1 week of exposure and 2 hrs/day [21]. Blood viscosity falling time reduced significantly after 4 weeks and 8 weeks of exposure in vivo [22].

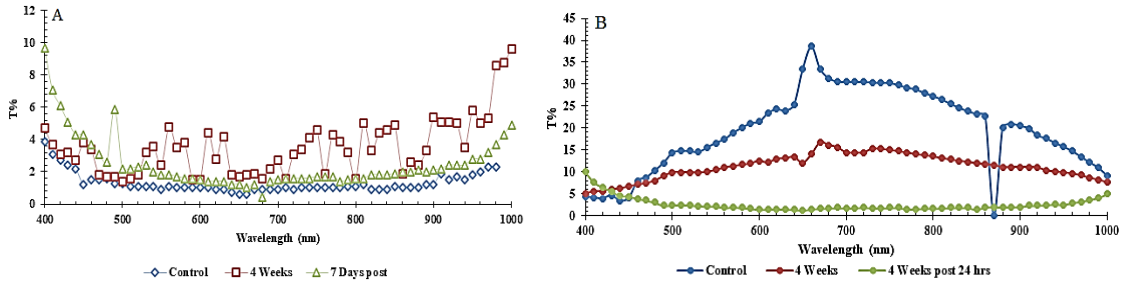

Figure 6 presents the alterations in blood transmittance that were recorded following a four-week exposure interval. (A) Blood specimens were subjected to an exposure duration of 4 hours per day for four weeks (square shape), with their transmittance evaluated 7 days after the conclusion of the final exposure (triangle shape). (B) Blood specimens were subjected to an exposure duration of 8 hours per day for four weeks (red circle), with their transmittance assessed 24 hours following the conclusion of the final exposure (green circle).

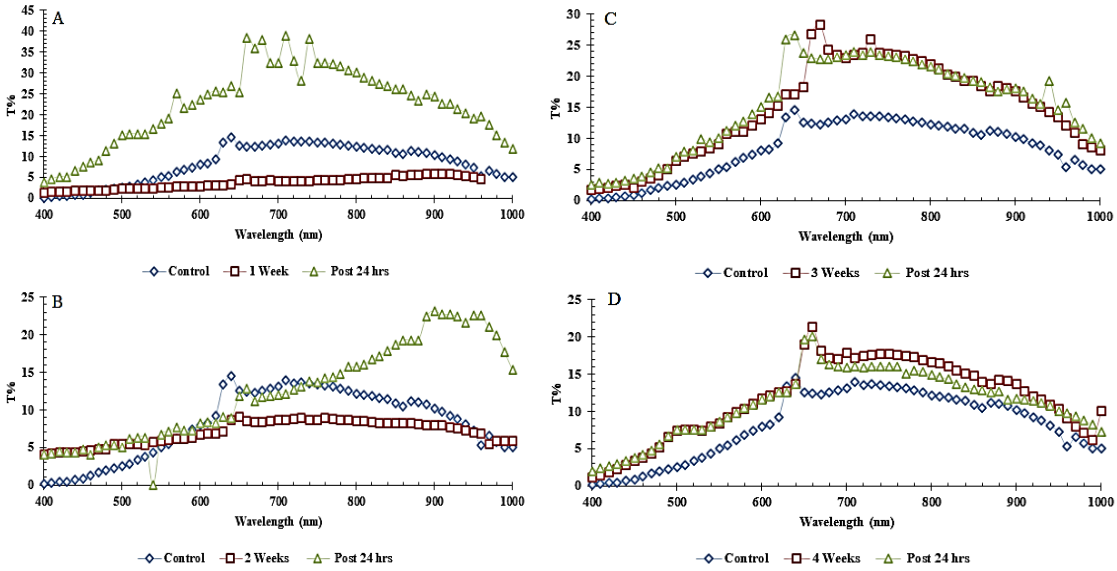

Figure 7: The transmittance property is shown twenty-four hours after exposure for 6 hrs per day during 1 - 4 weeks in A-D, respectively. T: transmittance

One research believes that the curve shift of transmittance of blood is strongly related to the RBCs orientation under the influence of SMF since RBCs' magnetic properties allow reaction with the external magnetic field [23]. The orientation will lower the energy state and align the cells parallel to the external field [24], [25]. Compared to our results, their in vitro results illustrated the drawback of transmission lines after exposure for a short period. In contrast, most of our post-exposure results were either comparable or enhanced at post-exposure. We believe that the key difference is the exposure duration since our in-vivo experiment applied a longer exposure period. This brings us to a conclusion where our exposure has left an irreversible effect on blood properties for some specific exposure periods. Magnetic properties of RBCs are related to a variety of factors and mainly the iron content of Hb, oxygenated status, negatively charged surface of the membrane, alignment and rotations, aggregations, and surrounding atmosphere (macromolecules, i.e., fibrinogen) of it [26]. Some of these factors stabilize the energy distribution and energy state of RBCs.

Optical properties of blood are one of the powerful techniques used in diagnostic and therapeutic purposes of blood [27], [28], [29]. It was shown that a 50% increase in Hct causes an increase in absorption and scattering, hence reducing the transmission of light. Additionally, the variation in oxygen status leads to a change in the absorption coefficient [30].

4. Conclusion

This study was investigating the effects of a 2.4 mT SMF on the transmission properties of blood. Among the various exposure durations examined, transmittance resulted a significant, nonlinear changes due to SMF exposures depending on the number of exposure weeks. Notably, exposures of one hour per day and six hours per day resulted in a remarkable shift in the transmittance peak, with a decrease or an increase of the blood's transmittance ability. These results indicate that extended exposure may facilitate the aggregation of red blood cells, thereby contributing to the observed fluctuations in transmittance, which is consistent with prior research. Conversely, exposure for 2 hours/day did not yield a substantial impact on the optical properties of blood.

Post-exposure time showed the remained effect of the SMF since the 24 hours post-exposure did not return the transmittance curve into the control value. However, the long post-exposure period returned the optical property of the blood to that of the control blood samples. We believe that the in-vivo

exposure performed a long-term, if not a permanent, impact on whole blood optical properties and hence blood components. The other parameters of blood content need to be examined under the influence of the magnetic field.

Author's Contribution

We confirm that all named authors have read and approved the manuscript. We also confirm that each author has the same contribution to the paper. We further confirm that all authors have approved the order of authors listed in the manuscript.

Conflict of Interest

There is no conflict of interest for this paper.

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