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Staining Studies of Anemic Human Blood Cells with Red Cabbage Extract: First Results

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Nilgün Güler Kuşculuo

Department of Chemistry Technology, Mustafa Cıkrıkcıoglu Vocational School, Erciyes University, Kayseri, 38039, Turkey

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ABSTRACT

The staining of anemic human blood cells by red cabbage (*Brassica oleracea*) extract has been investigated. Potassium aluminum sulfate (alum, $KAlSO_4{\cdot}12H_2O$), $FeSO_4$, $CuSO_4{\cdot}5H_2O$ or $K_2Cr_2O_7$ were used as mordants. Red cabbage leaves were extracted with distilled water at 100°C for 30 min and the liquid portion was separated from the precipitate by filtration. A two-drop blood sample was obtained from a 30-year-old male anemia patient. The sample was spread as a peripheral smear on ten glass slides and allowed to dry at room temperature. The slides were immersed into beakers, which contained red cabbage extract. Every extract included, respectively, alum, $FeSO_4$, $CuSO_4{\cdot}5H_2O$ or $K_2Cr_2O_7$. However, mordant was not added to one beaker. The beakers were kept in the oven at 50° C for 1 hrs. The staining procedure was repeated at100°C for one hour. The slides were then washed with distilled water, air-dried and viewed at 1000 magnification. As a result, we found that blood cells stained at high temperature and with mordant. Blood cells were not effectively stained without alum and at 50°C. However, different blood cells were stained with different mordants at 100°C for one hour in different colors. *Brassica oleracea* has the potential for use as a stain for studies of anemic human blood cells.

INTRODUCTION

It is known that several histological techniques, which are used to provide a nuclear stain, consist of natural phenolic compounds, which are structurally related to anthocyanins [1]. These include carminic acid (used in carmine dyeing) and hematoxylin as dye resources [2]. Anthocyanins in red cabbage and dahlia, hibiscus sabdariffa, Curcuma longa have also been used at histological dyeing [3, 4]. At the same time, red cabbage dye is a natural source used mainly as a food dye. Most naturally, occurring anthocyanins occur as a glycoside and contain one of the several aglycone cores [5]. Anemia is defined as the concentration of hemoglobin, which is below normal. The classification of anemia in our daily lives is based on the morphological structure of erythrocytes or the number of reticulocytes [6]. Since the number of reticulocytes is very important in evaluating the bone marrow response, their staining is also important. The reticulocyte count is reported as a percentage of circulating erythrocytes [7, 8]. Iodine, saffron, and ammonia carmine are suitable for staining tissues and cells, practically no advances have been made to our knowledge about the morphology of blood cells [9]. Recently, red blood cells have examined by a selective staining method and stained with synthetic dyes such as Giemsa [10, 11]. However, we could not find any studies about dyeing of anemic blood cells with natural sources. As a result, we hope that this study will be the first in this direction.

MATERIALS AND METHODS

All the common laboratory chemicals used in the synthesis of the substance were purchased from commercial sources and used without further purification. The red cabbage was obtained from Bafra (Samsun, Turkey). A blood sample was taken from 30-year-old male and anemia patient in a hospital of Erciyes University.

Extract preparation

A sixty-gram amount was taken from red cabbage leaves and ground to a dark blue-purple powder using grinding machines, then powder was taken in 300 mL distilled water. The solution was kept on a simmer half an hour and then, it was distilled. The liquid extract was waited in the cold place at 4°C to use for dyeing.

Peripheral blood film

The slides were cleaned with 70−90 % alcohol and allowed to dry. The middle or ring finger was selected to make a hole. The area was cleaned with 70% ethyl alcohol and punctured. A drop of blood was put on the clean slide. Blood was spread on the glass slide by another slide. This was repeated with several slides. Slides were dried and fixed with methanol [12].

Blood cells staining

A 300-ml extract of the plant was divided into six portions containers in the same quantity. No mordant was added to one container.0.5 g alum, $KAlSO_4 \cdot 12H_2O$, $FeSO_4$, $CuSO_4$, $K_2Cr_2O_7$were put into the other five containers and they were stirred with the baguette. Each slide was submerged into each container. Containers were put into the oven at 50°C for one hour. After one hour, slides were taken from containers, washed with water and left to dry. In this way, the dyeing process was completed. This process was repeated at 100° C for 1h. Photographs of blood cells on the slides were taken at 1000 magnification by light microscopy.

RESULTS

Photographs of the blood cells on the slides were used to observe their staining. These photographs are shown in figures below. Different anemic blood cells were stained in red cabbage extract with alum, copper sulfate, potassium dichromate and ferrous sulfate at 100˚C for 1 h. The microscopic examination results are shown in Figure 1a, b, c, d. Eosinophil, neutrophil, plasma and basophile cells, in particular, were stained blue-purple color in red cabbage extract with $FeSO_4$. Reticulocytes, plasma, and platelets were stained a yellow-green color with $CuSO_4$. Reticulocytes, thrombocytes were stained a blue-purple color with $K_2Cr_2O_7$. Platelets were stained as blue-green color with alum. We saw that dyeing's at 50° C without mordants did not succeed. Therefore, we have not given any photographs of them.

Microscopic photographs of anemic blood cells stained with Giemsa and red cabbage extract are shown in Figure 2a, b. Here, the photograph of the cell dyed with iron sulfate in red cabbage extract was used for comparison purposes. Because it contains more dyed blood cell components.

DISCUSSION

Anemia occurs when a person's blood has a lower than the normal number of red blood cells or if your red blood cells do not have enough hemoglobin. Normal anemia is defined by low erythrocyte count. Therefore, erythrocytes need to be made visible by dyeing. Three blood cells, such as erythrocytes, leukocytes, and thrombocytes are generally low in anemic patients [6]. Thus, staining of leukocytes and platelets will facilitate the counting process.

In this study, shaped elements such as psylliocytes, spherocytes, echinocytes, elliptocytes, and ovalocytes were found to be stained in red cabbage extract with different mordants at 100° C. Nowadays, anemic cells are counted in oncology and pathology laboratories by dyeing with synthetic dyes such as Giemsa. This is used to diagnose the pathology of the anemia patient. Although we have conducted work with different mordants and red cabbage extracts, no studies were found on the use of natural dyes in the dyeing of blood cells.

Many kinds of cell elements were dyed in red cabbage extract by only iron sulfate mordant. Therefore, violet-blue dyeing close to Giemsa dyeing was carried out with red cabbage extract containing iron sulfate at 100° C. Cell in less number was also stained with other mordants. At higher temperature, we could not examine denatured cells. Effective staining was not observed in cell elements at 50° C and without mordant.

In particular, we found that effective dyeing of neutrophils in anemic blood was not obtained in staining studies done at 50 or 100 degrees with mordant or without mordant in red cabbage extract. We hope that the use of different plant extracts at higher temperatures, and in different pH medium than in dyeing studies with a healthy, non-anemic human blood sample will give more positive results. There was the little study about dyeing of cells with plant extract. Also, no study has been conducted on the dyeing of blood cells by red cabbage extract and we aimed to compare the cell color of anthocyanin dye in red cabbage extract.

As a result of staining studies we have conducted, we believe that red cabbage extract in different mordants may be used to dye some anemic blood cell elements, instead of Giemsa which is a eukaryotic cell dye.

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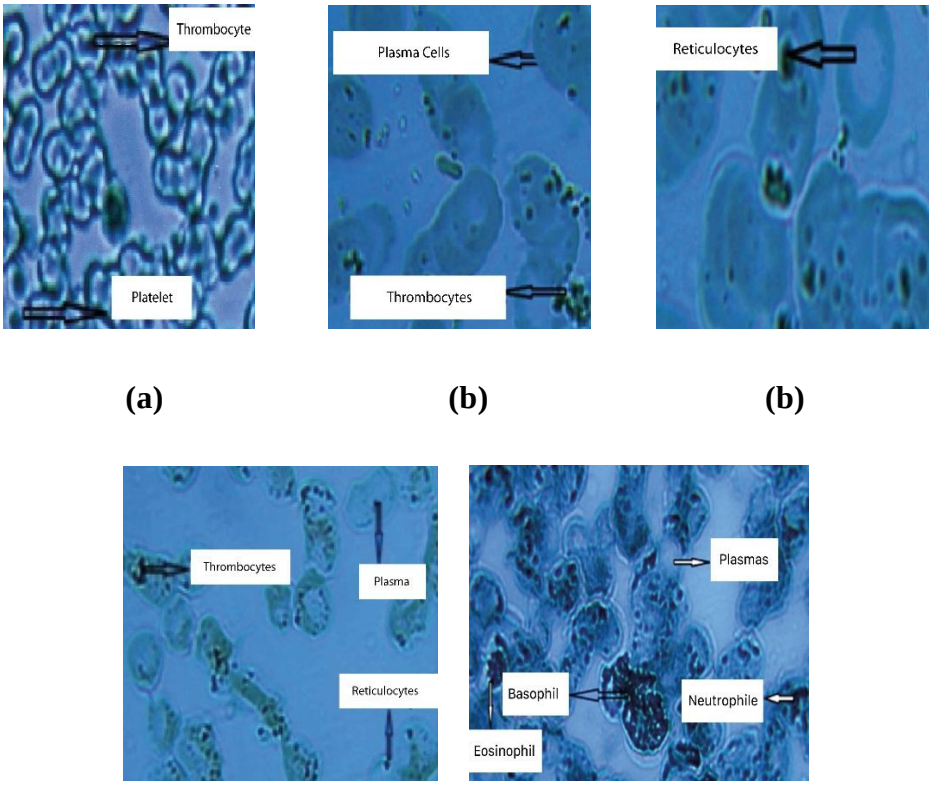

Figure 1. Photographs of different anemic blood cells stained with different mordants in red cabbage extract X100 (a) With alum, (b) With $CuSO_4$, (c) With $K_2Cr_2O_7$, (d) With $FeSO_4$

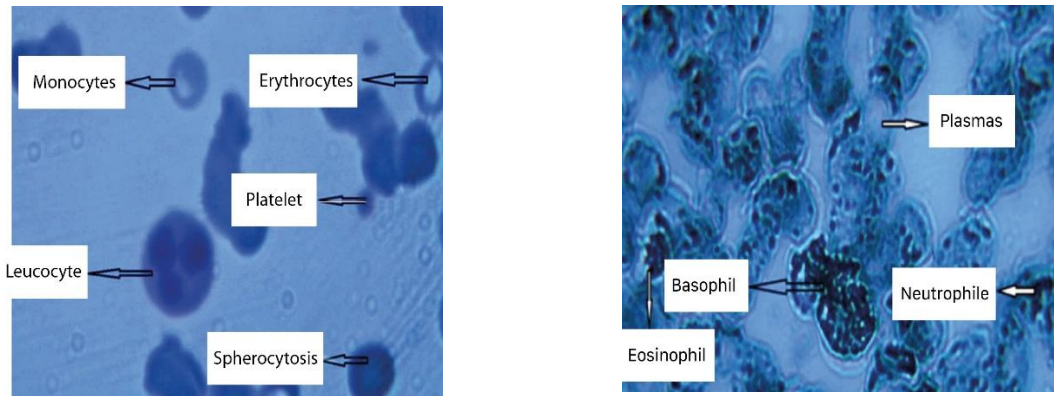

Figure 2. Photographs of anemic human blood cells were stained X 100 (a) with Giemsa (b) With $FeSO_4$ in red cabbage extract