

Staining Effect of Pomegranate Flower Extract on Human Blood Cells: First Results

Nilgun Guler Kusculuo and Huseyin Benli

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

Abstract: Pomegranate flower plant, popularly known using for the treatment of various diseases, was not investigated as a source of dye for cytological studies using human blood cells. The importance of this study is to appear dyeing result of pomegranate flower extract on human blood cells. The natural dye source was pomegranate flower known as roselle and potassium aluminum sulfate (alum = $\text{KAlSO}_4 \cdot 12\text{H}_2\text{O}$) was used as mordant or metal salt. Distilled water was used as solvent. Fresh, clean and air-dried flowers were extracted with distilled water at 100 °C for 30 minutes and then filtered. One drop blood from a healthy 20-year woman was spread as a peripheral on to ten plates and dried at nearly 25 °C. These slides were stained by soaking in pomegranate flower extract with/without alum ($\text{KAlSO}_4 \cdot 12\text{H}_2\text{O}$) at 100 °C for 60 minutes. Slides were washed with distilled water, dried and done microscobic examination. The different blood cells dyed dark orange in alum mordant media at 100 °C. As a result, pomegranate flower has the capacity to use dyeing human blood cells such as eosinophil, basophil and neutrophil.

Key words: Blood, neutrophil, pomegranate flower, basophil, eosinophil.

1. Introduction

Plants, insects or soils are used as natural dye sources [1]. The presence of non-hazardous natural dyes has supported increased environmental awareness so that the use of danger synthetic dyes decreased [2]. Nowadays, there are a few histological methods. In these methods, staining of cell components is done by some dyes which have natural aromatic structure like anthocyanins. Authors can give example, carmin and hematain stains as dye resources [3]. Anthocyanins from red cabbage, dahlia, hibiscus sabdariffa and curcuma longa were also used at histological stainings [4-6]. In addition, roselle is common used by medical researchers in the world. Especially, hypertension, pyrexia and liver damage are treated by means of roselle plant [7, 8].

Red blood cells which are taken from exact blood of matured mammalian have no nucleus. They have transfusion features.

Transfusions are used to treat hemorrhage and to

give oxygen to tissues. Clinical condition of patients is very important in transfusion of red blood cells [9, 10].

Tissues and cells dyed by Carmine have been known, but there are no advancements about structure of blood cells [11]. Red blood cells are examined by a selective staining method and stained with synthetic dyes such as giemsa [12].

This study, therefore, aimed at the use of pomegranate flower aqueous extract for the staining of human blood cells to investigate its ability to elucidate the blood cellular components.

2. Material and Methods

Pomegranate flowers were purchased from local market in Talas (Kayseri, Turkey).

2.1 Preparing Extract from Plant

A twenty-gram amount of dried flowers was ground to a dark red powder using grinding machines, then powder was taken in 100 mL distilled water. Solution was kept on a simmer half an hour and then it was distilled. Extract is waited in the cold place at

Corresponding author: Nilgun Guler Kusculuo, Ph.D., research fields: medicinal biology and biotechnology.

4 °C to dye.

2.2 Preparing Peripheral Blood Film

The slides were cleaned with 70 to 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 touched to the clean slide. Blood was spread on the glass slide by another slide. It was repeated with several slides. Slides were dried and fixed with methanol. If blood does not well up, the finger should be gently squeezed [13].

2.3 Dyeing of Blood Cells

A 120-mL extract of the plant was divided into two containers in the same quantity. Anything was not added to one container. 2.0 grams alum was put in to the other and it was stirred with baguette. Each slide was submerged to each container. The dye solution was put into oven at 100 °C for an hour. After an hour, slides were taken from containers, cleaned by water and left to dry. Photographs of blood cells on the slides were taken at 10 × 100 magnification by light microscopy.

3. Results

Photographs of the blood cells on the slides were taken for observation of their staining. Different cells

in blood were stained with pomegranate flower extract in alum, non-alum media at 100 °C. This was evident from the microscopic examination. Generally, blood cells such as neutrophil, basophil were stained at orange-brown colour. Same cells were stained with giemsa dye. The best staining quality was obtained with hibiscus in alum, non-alum media at 100 °C. Figs. 1 (a, b, c) have revealed staining quality.

4. Discussions

Well-stained blood film is very important for right cell structure assesment. There are some tried staining techniques like Leishman and Giemsa to dye various smears [14]. Authors' work is upon trying of a plant dye in blood cells. Some studies which include plant dyes are showed comparison of synthetic dyes with natural dyes [15].

Pomegranate flower stained the nuclei of the neutrophils, basophils deep orange-brown and can be considered a neutral dye, because, pH of human blood is nearly neutral. Dyeing of cells depends on the only interaction or bonding between dye molecule and cell. The staining colour of cell is effected by cell medium. If the cell color later staining is pink or blue, dye molecule or cell medium can be basic or acidic. Acetic acid was added to the dye bath. Colour of neutrophil or basophil cells in blood could be different from orange-brown.

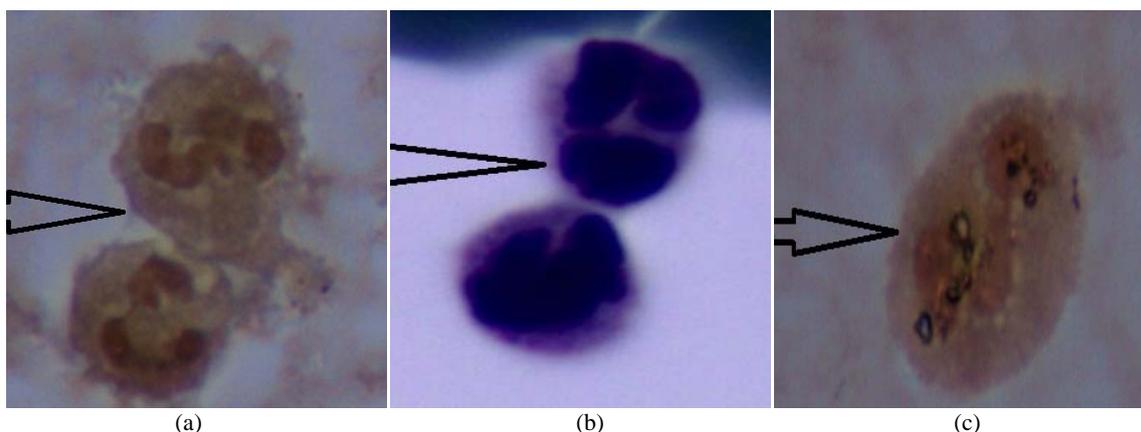


Fig. 1 Photographs of different blood cells stained in (a) pomegranate flower extract without alum; (b) giemsa dye solution; (c) pomegranate flower extract with alum.

In conclusion, pomegranate flower stain is natural, environmentally friendly and effective with ease of extraction from cheap plant source. Hibiscus (roselle) plant kinds commercially have produced by different countries that supply most of the requests of other countries [16]. If staining conditions are developed by hibiscus and pomegranate flower, it may be commercially produced in large quantity to be used as routine biological stain to replace the costly and imported synthetic dyes like giemsa and leishman in the nearest future. However, it is strongly recommended more elaborate studies should be carried out to fully establish the efficacy of the dye on different human and animal blood samples.

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