USING ALKANET (*Alkanna tinctoria)* EXTRACT at HISTOLOGICAL STAINING of WISTAR RAT KIDNEY TISSUE

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ABSTRACT

Natural dyes are obtained from three different sources: plants, animals and minerals. These dyes have been widely used in the textile, food and pharmaceutical industries for a long time. However, studies of evaluating the extracts obtained from plants as histological stains have been conducted recently. In this study, the ability of the with mordant and without mordant extract obtained from Alkanna tinctoria plant to stain tissues taken from wistar rat kidney was investigated. For this, 20 g of powdered plant was filtered with Whatman No 1 filter paper after being kept overnight at room temperature in 200ml 70% Ethyl alcohol. The filtrate was divided into 4 parts, one of which was left blank, and 3 grams of $CuSO_4$, $NiSO_4$, $KAlSO_4.12H_2O$ (alum) mordants were added to the others. After solubility of the mordant, 4 separate unstained preparates were immersed in the solutions after the paraffin was removed. The solutions were kept in the oven at 60°C for 1 hour. After the preparates were washed with distilled water, they were dipped in xylene and closed with entellan. 40 × images of tissues were taken under the light microscope. With the help of extracts from a plant, different colors were stained in the cytoplasm of epithelial cells in kidney tissue with different mordant substances

Key words: Alkanna tinctoria, kidney tissue, mordant, natural staining, alkanet

Introductıon

Natural dyes are environmental friendly. They exist in a lot of plants (1). According to their sources, dyes are divided into two classes as natural and synthetic (2). Dyes from plants in which they are completely or at least partially soluble are applied to different materials like textiles, leather, paper, hair, food, cells at different temperatures and time (3-6). For example, *Alkanna tinctoria,* is widely been in Europe and Western Asia (7). Phytochemicals are organic substances found in many plants such as *Alkanna tinctoria*. In recent studies, it is stated that phytochemicals protect people against various diseases (8). There are studies on the phytochemical analysis of colored extracts, especially obtained in methanol (9). *Alkanna tinctoria* is used in the treatment of skin wounds, diseases, and possessed antimicrobial activity (10). Also, this plant contains different naphthoquinone derivatives like shikonin, alkannin. These are color pigments to use staining studies (11-14). Studies have been conducted on the antibacterial effect of *Alkanna tinctoria* against various bacteria in aqueous and ethanol extracts, and more successful results have been obtained with ethyl alcohol

extract rather than aqueous extract (15). Although the biological activities of the colored substances contained in plants are the most studied subject, interest in staining different cells and their elements is also increasing. For example, Light purple staining of testes and ovary tissues of Wistar rats were made with Punica granatum flower extract (16).

This study aimed to emphasize the importance of using mordant to achieve purple and pink staining in tissue staining since there is no vivid, and good color staining on the cell cytoplasm by alkanet extract without mordant.

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**.** Dried *Alkanna tinctoria* were obtained from local markets of Kayseri province.

Tissue Supply

In this study, 2 Wistar albino rats, one female and one male, weighing 150–250 g and raised at the Erciyes University Experimental and Clinical Research Center (DEKAM) were used. This study was conducted with the ethics committee number 16/144 dated 16 November 2016. The rats were kept in cages, at 21 °C with normal daily organization, 12 h of light/dark, and fed and watered appropriately. The rats were put to sleep with ketamine and xylasine and their tissues taken, so their lives were ended. All of the procedures were performed according to ethical guidelines (18).

Deparaffinization of tissue preparates

Paraffin was removed from the preparate by the classical method. Preparates were left in an oven at 60 °C for one night. The next day they were removed from the oven and allowed to cool to room temperature for 3–5 min (18).

Preparation of *Alkanna tinctoria* extract

Alkanna tinctoria was ground to a dark black-blue powder using manual grinding machines (Waring, commercial). The dried powder of the plant weighed 20 g (Shimadzu bl 3200). The powder waited in 200mL 70 % ethanol (1:2) for 24 hours at room temperature. Then the extract was filtered two times with Whatman filter paper. The filtrate was boiled until half of the water had evaporated. The remaining blue-purple colored filtrate was used as the dye solution. The filtrate was stored in the refrigerator at 4°C and then was used for staining.

Dyeing of the tissues with *Alkanna tinctoria* extract

200 ml of Alkanet extract solution was equally divided into the four staining jars. Alum, CuSO4, NiSO4 mordants were added to one each of the staining jars. Only one was left empty. Preparates of kidney tissues were immersed in each extract solution. The jars were left in a 60 °C oven (NUVE 500) for 1 h. The preparates were taken from the solution and washed with a large amount of distilled water. The preparates were allowed to stand in 100%, 96%, 80%, and 70% ethyl alcohol for 5 min (for dehydration). They were closed with entellan after they were immersed in xylene for 3 min. The prepared slides were examined under 40 × magnification with a light microscope. The staining procedure was applied to the same tissues of two different rats under the same conditions, but since there is no significant difference in the staining results, the results of staining of the tissues of a single-sex are given in this study (18).

Results

Photographs taken under a light microscope of kidney tissue stained with extracts obtained from the alkanet plant were examined. In the staining done without adding mordant to the extract, pale light yellow-brown staining in the cytoplasm is shown in Figure 1. When added $CuSO_4$ mordant dark pink-purple color dyeing is shown in Figure 2. Dark brown coloring when staining by adding Alum is shown in Figure 3, and also $NiSO_4$ is added light purple dyeing is shown in Figure 4.

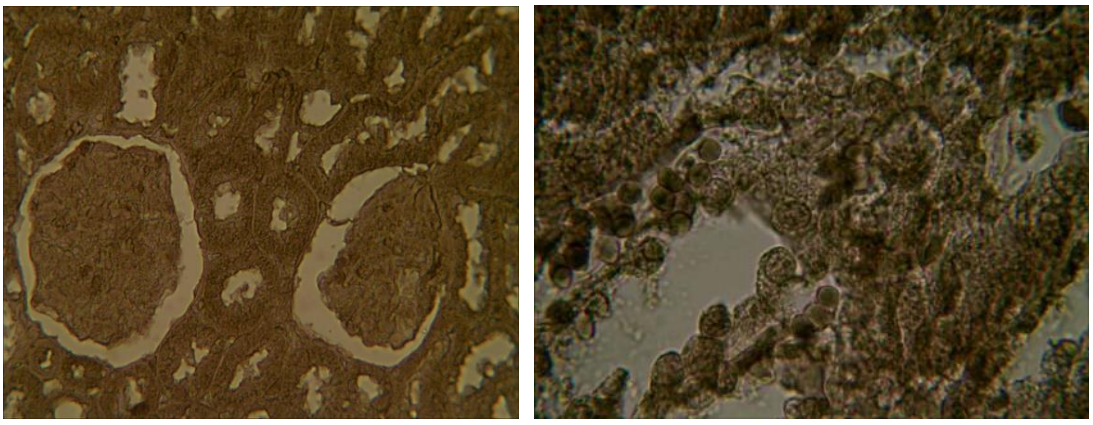


Figure 1. Photographs of stained kidney tissue without mordant

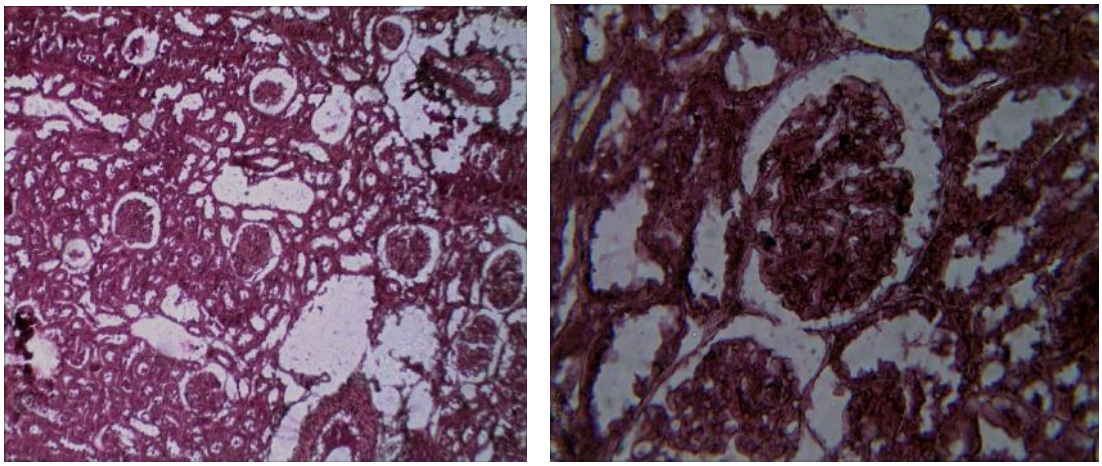


Figure 2. Photographs of stained kidney tissue with $CuSO_4$ mordant

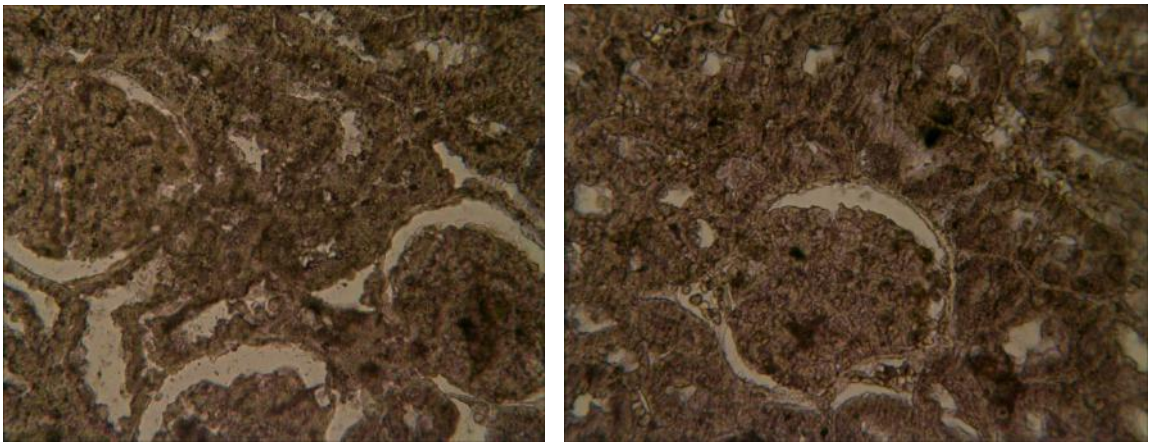


Figure 3. Photographs of stained kidney tissue with Alum mordant

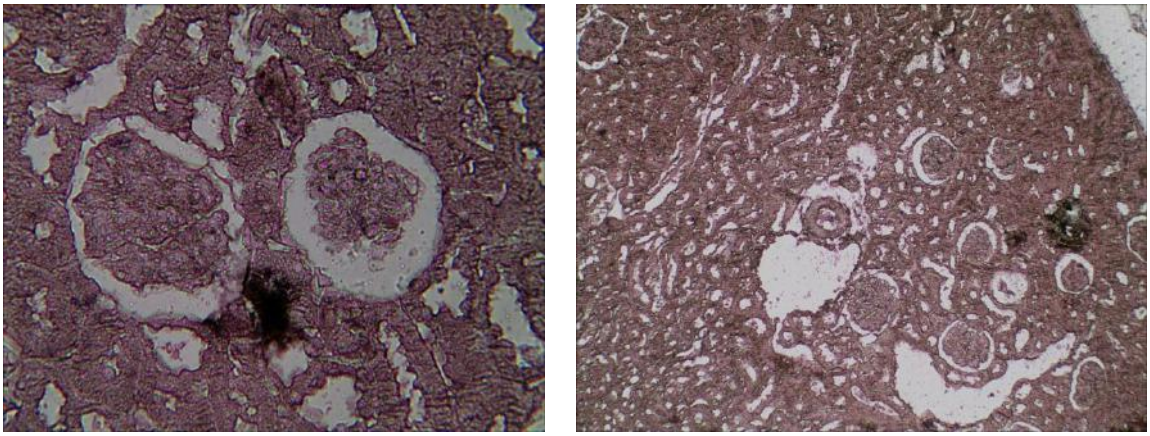


Figure 4. Photographs of stained kidney tissue with $NiSO_4$ mordant

Discussion

In a study conducted by Tung et al., After the isolation of the naphthoquinone groups in the structure of the *Alkanna tinctoria* plant, its antiproliferative effect was investigated, and a colon cancer cell line was used for this. Cell cycle profile and cell apoptosis have also been investigated by the flow cytometry method (17). Similarly, there are many studies on the biological activity of this plant. In a study conducted by Kuşçulu and Aslan, staining was performed on the cytoplasm of rat kidney tissue with *Punica granatum* flower extract (18). However, there are no tissue studies previously stained with the extract of the *Alkanna tinctoria* plant. It constitutes the original side of this study. In this study, the necessity of using mordant in cell staining with *Alkanna tinctoria* was revealed, since there is yellow-brown staining in the cytoplasm without mordant and more vivid dyeing with other mordants. Using mordant is important like $CuSO_4$ and $NiSO_4$ for staining in light or dark purple-pink.

References

1. Hu YL., Luo JY., Hu SR., Fu H., Yang SH., Yang MH. (2017). Application of natural plant pigments in enlarged health industry. U.S. National Library of Medicine, 42(13),2433-2438.
2. Nagar KH., El Sanad SH., Mohomed AS., Ramadan A. (2005). Mechanical Properties and Stability to Light Exposure for Dyed Egyptian Cotton Fabrics with Natural and Synthetic Dyes. Polymer-Plastics Technology and Engineering, 44(7),1269-1279.
3. Zollinger H. (2004). Color Chemistry. Syhtheses, Properties, and Applications of Organic Dyes and Pigments, 3rd ed. John Wiley and Sons. New York, 544-545.
4. Guler KN., Benli H. (2017). Staining Effect of Pomegranate Flower Extract on Human Blood Cells: First Results. Journal of Environmental Science and Engineering A, 6(2),249-251.
5. Bassey RB., Bakare AA., Peter Al., Oremosu AA., Osinubi AA. (2012). Factors Influencing extract of Hibiscus sabdariffa Staining of Rat Testis. Biotechnic and Histochemistry, 87(6),403-407.
6. Chandra R., Babu KD. (2010). Propagation of Pomegranate- A Review. Fruit, Vegetable and Cereal Science and Biotechnology.Global Science Books, 4(2),45-50.
7. Papageorgiou VP. (1978). Wound healing properties of naphthaquinone pigments from *Alkanna tinctoria*. Experientia, 34, 1499-1501.
8. Jana P., Rahaman C., and Banerjee N. (2009). Pharmaco-anatomical and phytochemical studies of Plumbago zeylanica- an important medicinal plants. Advances in Plant Biology, 318-336.
9. Tıwarı A., and Sarıvastava M. (2018). Phytochemical analysis of Methanolic dye extracts of some medicinal plants. International Journal of Applied Home Science, 5(5-9),939-942.

10. Hasmida MN., Nur Syukriah AR., Liza MS., and Azizi MCY. (2014). Effect of different extraction techniques on total phenolic contents and antioxidant activity of Quercus infectoria galls. Internat.Food Res J, 21(3),1075-1079.
11. Papageorgiou VP., Winkler A., Sagredos AN., Digenis GA. (1979). Studies on the relationship of structure to antimicrobial properties of naphthaquinones and other constituents of *Alkanna tinctoria.* Planta Med, 35,56-60.
12. Papageorgiou VP. (1977). New pigment of *Alkanna tinctoria* having naphthaquinone structure. Planta Med, 31,390-394.
13. Papageorgiou VP., Digenis GA. (1980). Isolation of 2 new alkannin esters from *Alkanna tinctoria*. Planta Med, 39,81-84.
14. Assimopoulou AN., Sturn S., Stupper H., Papageorgiou VP. (2009). Preparative isolation and purification of alkannin/shikonin derivatives from natural products by high speed counter current chromatography. Biomed Chromatogr, 23, 182-198.
15. Alwahibi MS., and Perveen K. (2017). Chemical anlysis by GC-MS and in vitro antibacterial activity of *Alkanna tinctoria* extracts against sking enfection causing bacteria. Biomedical Research, 28(18),1-4.
16. Kuşçulu NG. (2018). Evaluation and comporison of staining effect of *Punica granatum* flower extract on testis and ovary of Wistar rats: First results. African Journal of Biotechnology, 17(32), 989-993.
17. Tung NH., Du GJ., Wang CZ., Yuan CS., Shoyama Y. (2012). Naphthoquinone Components from *Alkanna tinctoria* (L.) Tausch Show Significant Antiproliferative Effects on Human Colorectal Cancer Cells. Phytotherapy Research, 27(1),66-70.
18. Güler Kuşçulu N., Aslan HG. (2019). Evaluation of an extract of the *Punica granatum* flower as a biological stain of rat tissues: a preliminary study, Molecular Biology Reports,46, 581–585.