THE USE OF WATERY EXTRACT OF KUJARAT FLOWERS HIBISCUS SABDARIFFA AS A NATURAL HISTOLOGICAL STAIN

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Abstract

Background: Hibiscus sabdariffa is cultivated in many countries in the world, Iraq is one of these countries. Watery extract of kujarat flowers Hibiscus sabdariffa is red in color and acidic in taste. Previous investigations used watery extract of kujarat in medical studies and food industry. Others used its watery extract to stain blood film, fungi and plants. No previous studies use the watery extract of kujarat instead of eosin and together with hematoxylin stain in the routine H and E stain.

Objective: to see the possibility of using watery extract of kujarat as a natural histological stain and instead of eosin. This study proposed because of the similarity in some characters between kujarat and eosin stain.

Methods: watery extract of kujarat flowers was prepared in 20% concentration weight/volume. It was used to stain tissues from albino mice. It was used instead of eosin stain in the ordinary hematoxylin-eosin stain.

Results: Stained tissues reveal acceptance to kujarat stain. Erythrocytes, cytoplasm of epithelial cells of kidney tubules and smooth muscle fibers appear brownish in color. Nuclei of stained cells appear dark violet in color.

Conclusion: watery extract of kujarat flowers need chemical purification to separate its acidic from basic components. This study proposes the use of purified acidic part with the pigment instead of eosin. This part will have closer characterization (physical and chemical) to the eosin stain.

Keywords: Hibiscus sabdariffa, Roselle, kujarat flowers, natural histological stains.

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Introduction

Hematoxylin-eosin stain is the most important routine histological stain that has been used in most laboratories. It is combination of the natural hematoxylin stain and the synthetic eosin stain. Hematoxylin is a basic dye that stains acidic components of the cell, eosin is an acidic dye that stains the basic cytoplamic components of the cells. Nuclei of the cells take the hematocylin dye and appear dark violet or blue in color, cytoplasm of some epithelial cells, erythrocytes take eosin dye and stain pink[1].

Kujarat flowers *hibiscus sadariffa* belong to the family Malvaceae, which is commonly called roselle. Roselle is cultivated in India, Malaysia, Tropics, subtropics and central America[2,3]. Egyptians use to drink kujarat extract and call it karkadae, while in Iraq, it is called red tea. Studies in Iraq suggest using of kujarat extract in food industry, using it as a syrup and coloring agent[4]. Kujarat was found as a natural source of pectin, which solidify jelly and ice cream preparation[3,5].

Kujarat calyces contain per 100gm of edible portion, calcium (1.263mg), niacian (3.765mg), riboflavin (0.277mg) and iron (8.98mg)[2]. Chemical analysis in Iraq[5] of kujarat reported their values of 100mg. Of Ca and 9.55mg of Fe per mg of dry matter. Three water-soluble ploysaccharids have been isolated from flower buds of hibiscuse sabdaiffa (HIB, 1.2.3)[6].

Medical uses of these flowers are wide. Infusion of calyces are regarded as diuretic, choleretic, febrifugal, hypotensive, decreasing the viscosity of blood and stimulating intestinal peristalsis[2]. Other medical study proposed its effect in protection from induced cytotoxicity and genoetoxicity by different mechanisms[7].

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Other researchers proposed its use as a natural stain. They used extract to stain blood film, fungi and plant tissue[8-10].

Dried calyces of kujarat have dark red pigment. Chemical components of this pigment was reported in previous studies. It contains flavonoids gossypetine, hibiscetine and sabdaretine. The major pigment reported as hibiscin, that was identified as daphniphylline. Small amount of delphinidine 3-monoglucosides, cyanidin 3-monoglucosides (chrysanthenin) and delphinidine are also present[2].

This study is proposed because of the easy extraction of the pigment of kujarat flowers by boiling them in water. Other reasons is the wide cultivation of these flowers in Iraq. Dried flowers can be easily obtained from local markets and are cheap in price. The acidic taste of the kujarat extract is considered as another reason as compared with acidic eosin stain.

Materials & Methods

Dried flowers of kujarat were obtained from local market. They were washed with water to clean them from dust, boiled in water to prepare the extract in 20% concentration (weigh/volume) by boiling 20mg of dried flowers to 100ml water for minutes. The extract were filtered and kept in clean dried bottle at room temperature for 2-3 days. Few crystals of thymol were added to prevent fungal growth. The extract can be kept refrigerator for months[10]. Higher than 20% concentration of the extract results in solidification of the extract. PH of kujarat extract was calculated by PH meter to see its degree of acidity.

Two types of tissue were selected. These tissues are kidney tubules and muscular wall of blood vessels. That selection based on the basophilic components of these tissues and their possible ability to accept kujarat stain. Slices from kidney and medium sized artery of albino mice were prepared by the routine histological procedures[1,11]. Slides were stained by hematoxyline kujarat stain by the proposed steps:-

- two steps of xylene 5 minutes each.
- Dehydration by ethyl alcohol from absolute to 95% - 70% - 50%.
- Stain with hematoxylin 10 min.
- Running water 10 min.
- Dip in acid alcohol.
- Running water 10 min.
- Stain with kujarat extract 10 min.
- Wash with running water.
- Dip in xylene.
- Used D.P and cover the tissue and examine.

The prepared stained slides were examined by light microscope for evaluation and interpretation of results.

Results

Result of PH calculation of extract is 2.39. Result of preparation of watery extract of kujarat reveals solidification of the extract in higher concentration than 20%. Other results revealed that animal tissues have apparently reacted with the stain of kujarat when it is used instead of eosin. Interpretation of these findings in the accompanied figures as following:-

Figure 1: Muscular tissues in the wall of medium sized artery. The figure shows cross section in muscular artery. The most prominent histological features is the internal elastic membrane. It is dark violet wavy line stained by hematoxyline dye. The middle layer, tunica media is the widest region and composed of circular lamellae of smooth muscle fibers. Cytoplasm of these cells stains faint brown. Other histological features is the faint brown coloration of red blood corpuscles inside the lumen of a blood vessel which support the later finding of[9].

Figure 2: higher magnification of muscular tissue in the wall medium sized artery, the figure shows muscular fibers with dark violet nuclei and cytoplasm is faint brown in color.

Figure 3: kidney tissue. The figure shows cross section in kidney cortex. It shows prominent dark violet nuclei and faint

brown cytoplasms in renal glommeruli and tubules.

Figure 4: Higher magnification of kidney tissue. The most prominent feature is the dark violet nuclei of epithelial cell of renal tubules and endothelial cells of glommeruli. The other feature is the faint brown cytoplasm of tubular epithelium. The narrow lumen tubules are the proximal convoluted tubules and the wide lumen tubules are the distal convoluted tubules.

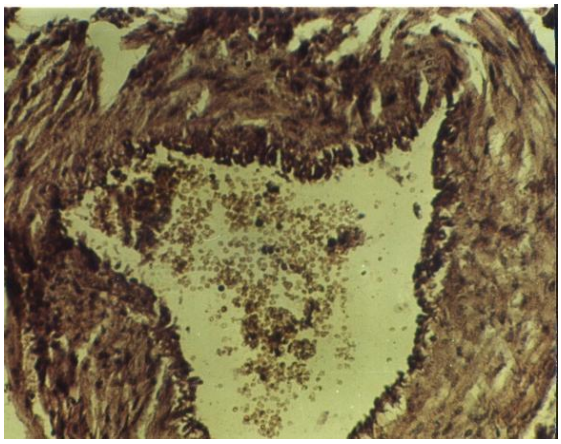

Figure 1: Cross-section in lung muscular artery. RBCs inside lumen stained faint brown. Hematoxylin & kujarat stain X50.

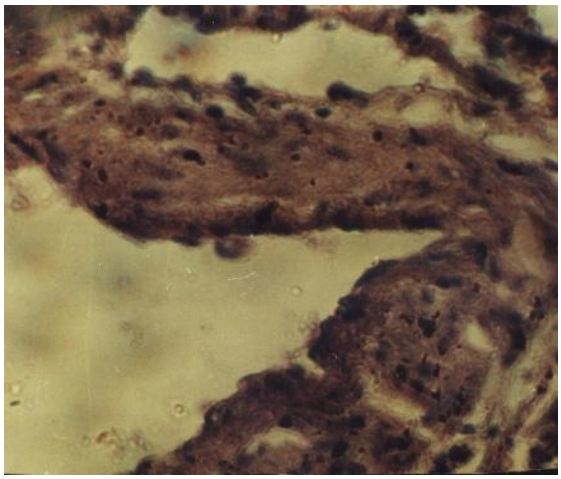

Figure 2: Higher magnification for Figure 1, nuclei of smooth muscle cells appeared dark violet, cytoplasm stain faint brown. Hematoxylin & kujarat stain, X450

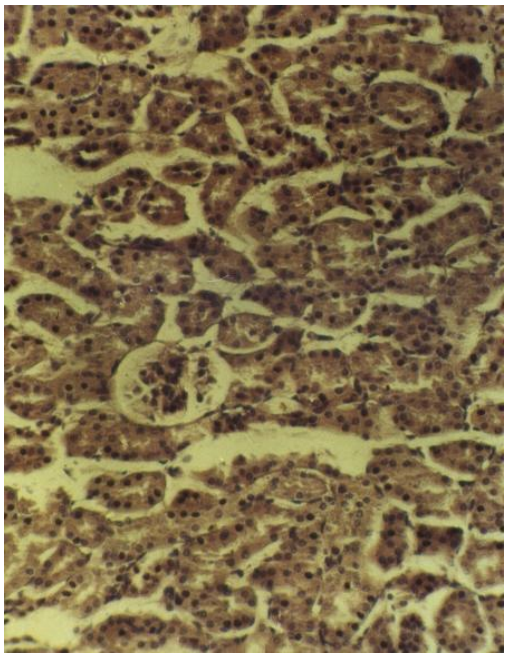

Figure 3: Cross section in kidney cortex, notice glommerruli and renal tubules hematoxvlin kujarat stain. X50.

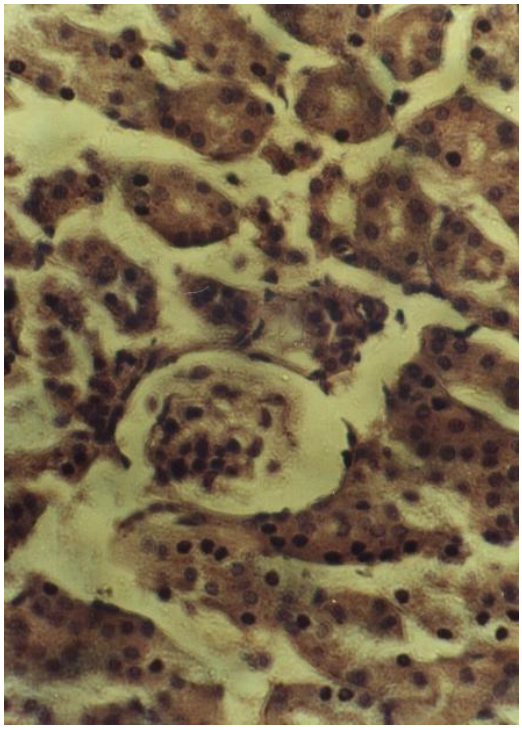

Figure 4: Higher magnification for Fig. (3) nuclei appeared dark violet. Cytoplasm stained faint brown. Hematoxylin and kujarat stain, X450.

Discussion

Result of PH calculation of the extract of kujarat is (PH = 2.39) that supports our proposed thought in using

kujarat instead of eosin as acidic stain. Previous studies proposed using of 20% conc. Of watery extract of kujarat in staining blood cells, fungi and plant cells[9,10]. We used the same concentration 20% weight/volume of kujarat extract in our study.

Previous studies didn't mention the risk of using higher than 20% conc. Of watery extract of kujarat. We noticed the extract will start to solidify as the concentration increase during boiling. That finding is based on the presence of pectin as suggested by[3,5]. Other results are the differences in color between the pigment of the extract of kujarat and the apparent color in slides after staining. The watery extract of kujarat is dark red and faint brown in tissues after staining.

Eosin is a synthetic dye. It is pure acid xanthene or phthalein pigment[1,11]. It stains specially the basophilic components of the cell[12-14]. Its watery or alcoholic solution appears bright, translucent orange in color. It stains cytoplasm of epithelial cells of proximal convoluted tubules of kidney, smooth muscle fibers and erythrocytes pink to red in color.

The watery extract of kujarat calyces is dark red in color, chemically it contains ascorbic acid, sugar (HIB 1,2,3) and other (Ca, Fe, niacin..etc)[2,5]. The chemical analysis is shown in table 1 and 2. According to these studies in Iraq and Guatemala, that natural fluid of kujarat has many chemicals associated with its pigments. These chemicals may alter its power of reactivity as tissue stain.

That chemical mixture may give a chance for many chemical reactions to occur during tissue staining and it may explain the difference in coloration between the extract and the stained tissue.

Our results in staining red blood corpuscles are in agreement with that results of[9]. Their study didn't discuss brownish coloration of these cells in stained blood film with kujarat.

This study proposed chemical separation of acidic part of kujarat extract. That purification may help the pigment to work more specifically with cellular components, and could result in close coloration between color of the kujarat extract with that of stained tissue.

Table 1: chemical analysis of Hibscus sabdarrifa in Iraq (Ali, 2000) chemical analysis for the kujarat caylces (dried) in Iraq.

Elements	Percent
Protein	1099%
Carbohydrate	71.93%
Moisture	5.65%
Ash	7.38%
Fibers	9.25%
Fat	3.80%
Element	mg/g of dried matter
Ca	100
Fe	9.55
K	120
Na	40
Mg	80
Cu	0.90
Mn	0.60
Ni	0.56

Table 2: chemical analysis of Hibiscus Sabdariffa (Rosselle) made in Guatemala (Morton, 1978). Food value per 100g of edible portion calyces, fresh of Hibiscus sabdariffa

Constituent	weight
Moisture	9.2 g
Protein	1.142 g
Fat	2.61 g
Fiber	12.0 g
Ash	6.90 g
Calcium	1.263 mg
Phosphorous	273.2 mg
Iron	8.98 mg
Carotene	0.029 mg
Thiamine	0.117 mg
Riboflavin	0.277 mg
Niacin	3.765 mg
Ascorbic acid	6.7 mg

References

1. Humason GL: Animal tissue techniques 3rd ed. W.H. Freeman and company sanfransisco.1972; p.p. 156-9.
2. Roselle MJ: Fruits of warm climates. 1987; p.p. 281-6. Miami, FL. FL.
3. Durance RD, Myers NF, and Roselle FL: ECHO plant information sheet. 1999; 17430.
4. Al-Zubaidy ZN, Baban HAK, and Flaij FKh: Treatments guide by medical Iraqi plants. 1977.
5. Ali WM: Chemical analysis and biological active of kujarat flowers Hibiscus sabdariffa L. and its use in ice-cream industry. MSc thesis, College of Agriculture, University of Basrah, 2000.
6. Muller BM, and Franz GL: Chemical structure and biological activity of polysaccharides from Hibiscus Sabdariffa. 1992.
7. Tesng TH, Kao ES, Chu CY, Chou FP, Lin Wu HW, and Wang CJ: Protective effect of dried flower extracts of Hibiscus sabdariffa L. against oxidative stress in rat primary hepatocytes. Food Chem Toxicol, 1996; 35 (12): 1159-64.
8. Al-Sarraj K, Zaman, A, and Lamya YAA: A new natural and simple stain for fungi. J Comm Med, 1997; 10 (1).
9. Al-Sarraj K: A new simplified stain for blood film using flowers of kujarat. J Comm Med, 1997; 10 (1).
10. Al-Sarraj K, Jawdat SZ, and Gaffer E: A new natural and simple stain for cell division. J Comm Med, 1997; 10 (1).
11. Bancroft JD, and Stevens A: Theory and practice of Histological techniques, London: Churchill Livingston.1977.
12. Junquera LC, Carneiro J, and Kelly RO: Basic Histology, 6th ed. A LANG. Medical book. Puentic hall international. 1989; p.p. 18-28.
13. Bajpai RN: Human histology 2ed ed. JAYBEE BROTHERS 85-A, Kamal Nagar, India. 1982; p.p. 41-8.
14. Young B, and Health JW: Wheater's Functional Histology, a text and color atlas: Churchill Livingston. 2001; p.p. 2-26.