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ASSESSMENT OF STAINING QUALITY OF ROSELLE (*hibiscus sabdariffa*) ON FORMALIN-FIXED PARAFFIN-EMBEDDED RENAL TISSUE SECTIONS

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ABSTRACT

Objective: This experimental descriptive study aimed to explore the efficiency and resolution of *Hibiscus Sabdariffa* (HS) as staining dye for renal histological sections as compared to Hematoxylin-Eosin routine stain.

Methods: Paraffin-embedded formalin-fixed tissue sections from kidney were stained by *Hibiscus Sabdariffa* solution using different concentrations and time durations in room temperature.

Results: Best results were obtained by using 5% HS solution for one hour. Bad results were noticed mainly when duration of staining was only 1-2 minutes.

Conclusion: Hibiscus Sabdariffa is an efficient natural cheap substitute of eosin in the ordinary stain H&E (Hematoxylin and Eosin) for histological sections.

Key Words: Hibiscus Sabdariffa, Histological Staining

INTRODUCTION

Histological stains have been important for better appearance of cells and tissues under the microscope to reach accurate and proper diagnosis [1]. Most of histological stains in current use are of synthetic origin; however, natural dyes are still promising to be cheaper potential sources for histological stains [2]. Any development of new histological stain is justified if the new stain is cheaper, available, harmless, and easier in application [3].

Hibiscus Sabdariffa (*HS*) is a plant belongs to the vascular flowering plants, known as Roselle or Red Sorrel in English and Karkade in Arabic [4]. Sudan is the largest country that produces and exports Karkade (*HS*) [5]. The plant has several uses; the outer thick red and fleshy cup-shaped leaves, for example, are commonly used in the production of several food products and are consumed worldwide as a cold beverage and as a hot (sour tea) drink [6].

The staining potentials of this plant were poorly explored; the current study, to our knowledge, represents the first initiative of using a pure aqueous extract of this plant in the staining of histological sections in Sudan.

MATERIAL AND METHODS

This experimental descriptive study was conducted during January 2014. A healthy kidney was obtained from a healthy rabbit, sliced and fixed in 10% formalin, processed through ascending grades of ethanol, cleared in xylene, and then embedded in paraffin wax. The specimen was cut into 160 of 3-5 μ m-thick sections. These sections were divided into four equal groups; each group was stained by a different concentration of the Hibiscus solution (1%, 5%, 10%, or 100%) in different time durations (1-2 minutes, 10 minutes, 30 minutes, and 60 minutes) at RT (room temperature). Nuclei were stained by haematoxylin stain and the cytoplasm and other struc-

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tures were stained by Hibiscus solution. Stained slides were then dehydrated, cleared, mounted with DPX, and assessed under a light microscope. The obtained data was analyzed by using Statistical Package for Social sciences (SPSS) software version 16 and Ethical Clearance was obtained from the Ethical Committee of Faculty of Medical Laboratory Sciences Neelain University.

Preparation of Hibiscus Sabdariffa extract and staining solution

A measured quantity of the Roselle powder (1 g, 5 g, 10 g, or 100 g) was brought to boil in 100 ml distilled water with continuous mixing and shaking; allowed standing for 30 minutes, and then filtered to obtain the colored extract with the appropriate concentration.

RESULTS

Quality of staining by Roselle was graded into 4 grades: bad, poor, good, and very good; that was according to the microscopic appearance of cell membrane, nuclear membrane, cytoplasm transparency, and extracellular matrix. If all the four parameters were clearly seen, quality was given the grade very good; if three were clearly seen, it was good; if two, it was poor; with only one parameter clearly seen quality was considered bad.

With 1% solution, no very good results obtained (Table No 1). With 5% solution, 5% of tissues showed very good staining (Table No 2). With 10% solution, 2.5% showed very good staining (Table No 3). With 100% solution, no very good results obtained (Table No 4). For all concentrations, the best time duration for better staining was 60 minutes.

DISCUSSION

Several research papers discussed the nutritional and therapeutic benefits of Hibiscus Sabdariffa [7, 8, and 9]. On the contrary, few researchers tried to apply extracts of the plant in diagnostic medical laboratory procedures. This study is a new one in the track of exploring laboratory properties of Karkade; pure water extracts without additions were used instead of eosin to stain cytoplasm and extracellular elements with encouraging results. The only disadvantage noticed was that a long time (one hour) was needed to obtain better results.

Eman A. Hashim [10] applied 20% concentration of Hibiscus Sabdariffa instead of eosin stain in the ordinary Hematoxylin- eosin stain to stain tissues from albino mice. She proposed the use of purified acidic part of Hibiscus Sabdariffa instead of eosin because this part has similar physical and chemical characteristics to the eosin stain.

Ihuma et al [11] reported that methanolic extracts from H.sabdariffa could be used as a staining agent for some fungi and therefore reduced the problems associated with over-dependence on toxic, expensive and scarcely available exotic stains.

Egbujo EC and colleagues [12] prepared Roselle (Hibiscus Sabdariffa) water extracts with various modifications and used it for the differential staining of rabbit testicular tissue sections. Various levels of differentiation of nuclear and cytoplasmic structures as well as other structures of this organ were obtained especially when 1% eosin was applied as a counter stain. The best staining result was obtained when iron alum was used to mordant the extract and when the extract mordanted with potassium alum was acidified using acetic acid and used to stain the sections. Modification of the aqueous extract to an alkaline pH using ammonia gave the poorest staining effect.

Benard Solomon [13] prepared a stain composed of H.sabdariffa extract, ferric chloride, sodium chloride, and glacial acetic acid. This solution was used to stain paraffin sections of formaldehyde-fixed tissues at 4 microns along with parallel Hematoxylin and Eosin (H&E) staining for control. Results showed that staining of nuclei with the extract solution was comparable with those sections stained with H&E. It was therefore suggested that the extract solution could be a progressive nuclear stain substitute for hem alum in H&E procedure due to its domestic availability, ease of preparation and use, resistance to fading and above all its good nuclear staining properties.

CONCLUSION

As a conclusion from this study, Hibiscus Sabdariffa is a cheap natural efficient staining dye for histological sections and it is comparable to the routine Hematoxylin and Eosin stain. However, larger studies with careful adjustment of temperature and time are highly recommended.

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Table 1: Results of staining by 1% Hibiscus Solution in different times

Bad	Poor	Good	Very Good	Time Duration	NO of Sections
9	1	0	0	1-2 min	10
8	2	0	0	10 min	10
8	1	1	0	30 min	10
6	3	1	0	60 min	10

Table 2: Results of staining by 5% Hibiscus Solution in different times

Bad	Poor	Good	Very Good	Time Duration	NO of Sections
7	3	0	0	1-2 min	10
6	4	0	0	10 min	10
5	3	2	0	30 min	10
3	3	2	2	60 min	10

Table 3: Results of staining by 10% Hibiscus Solution in different times

Bad	Poor	Good	Very Good	Time Duration	NO of Sections
9	1	0	0	1-2 min	10
8	2	0	0	10 min	10
5	4	1	0	30 min	10
4	3	2	1	60 min	10

Table 4: Results of staining by 100% Hibiscus Solution in different times

Bad	Poor	Good	Very Good	Time Duration	NO of Sections
6	4	0	0	1-2 min	10
5	5	0	0	10 min	10
4	4	2	0	30 min	10
4	3	3	0	60 min	10