Using of *Hibiscus Sabdariffa* extract as a natural histological stain of the Skin

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ABSTRACT

Objective: this experimental comparative study aimed to explore the efficiency of *Hibiscus Sabdariffa* as natural staining dye for skin tissue histological sections as compared to the routinely and commonly used stain, hematoxylin and eosin.

Methods: paraffin-embedded formalin-fixed tissue sections from skin were stained by different concentrations of *Hibiscus Sabdariffa* solution in different times at room temperature and compared to H&E-stained sections.

Results: the best results were obtained when 5% solution was used. The best time duration for staining was 60 minutes.

Conclusions: *Hibiscus Sabdariffa* solution can be used as a cytoplasmic stain instead of eosin in the H&E method to stain formalin-fixed paraffin-embedded skin tissue sections.

Key Words: Hibiscus Sabdariffa, Histological Staining, Skin

{**Citation:** Esam M. A. Raheem, Abd-Alhafeez Osman Ibnouf, Osman Hashim Shingeray, Hamza JM Farah. Using of *Hibiscus Sabdariffa* extract as a natural histological stain of the skin. American Journal of Research Communication, 2015, 3(5): 211-216} www.usa-journals.com, ISSN: 2325-4076.

INTRODUCTION

Development of new histological staining method remains justified especially if the new stain is harmless, easy to use, cheap, commercially favorable, and gives the desired practical results [1]. The most common and popular stain in histopathology laboratories is Hematoxylin-Eosin stain (H&E). The Hematoxylin stains the acidic components of the cell (nucleus), giving them dark violet or blue color. The Eosin stains the basic components of the cell (cytoplasm), giving them pink color [2].

Hibiscus Sabdariffa is a plant cultivated in many countries in the world; Sudan is one of these countries [3]. In Sudan, it is called Karkade; the extract is usually used as a drink, hot tea-like in winter and cold in summer. Watery extract of Hibiscus Sabdariffa is red in color and acidic in taste. The plant has several well-known industrial, medical, and nutritional uses [4, 5, and 6]. Little research explored the staining potentials of Hibiscus Sabdariffa as a substitution of eosin in H&E staining method [7, 8, and 9].

To our knowledge, the current study represents the first initiative of using an aqueous extract of this plant in staining of skin tissue sections in Sudan.

MATERIAL AND METHODS

This was an experimental comparative study conducted during a three-month period at the histopathology laboratory of Ahlia Portsudan College. The material of the study included skin tissue biopsies obtained from the department of dermatology of Portsudan Teaching Hospital. These specimens were fixed in 10% formalin overnight, and then processed at the histopathology laboratory to make paraffin-embedded tissue blocks. From these blocks, 160 of 4 μ m-thick tissue sections were obtained and made into 4 equal groups. The first group of sections was stained by 1% Hibiscus solution, the second group by 5%, the third group by 10%, and the fourth group by 100% solution. Sections in each group were divided into 4 equal sub-groups, the first stained for 2 minutes, the second stained for 10 minutes, the third stained for 30 minutes, and the fourth stained for one hour. Additional 10 sections were obtained and stained by H&E stain as control. Note: Hibiscus solution replaced eosin in H&E staining method as cytoplasmic stain. All staining procedures were in room temperature (25-30 °C). *Hibiscus Sabdariffa* staining solution was prepared as previously described [8]. The obtained data was analyzed by using SPSS

software version 20 and Ethical Clearance was obtained from the Ethical Committee of Faculty of Medical Laboratory **S**ciences Neelain University, Sudan.

RESULTS AND DISCUSSION

Quality of staining in histology depends largely on the microscopic appearance of cell membranes, nuclear membranes, cytoplasm transparency, and extracellular matrix [Ref]. If all are clearly seen without significant artifacts, quality is considered excellent. Quality is graded into good, poor, or bad if there is defect in appearance of one, two, or three of the four mentioned cell components, respectively.

With 1% Hibiscus solution, in this study, no excellent or good results were noticed. Bad results were the majority (table 1).

Table (1) Show results of staining with 1% Hibiscus solution in different times

NO	Time	Excellent	Good	Poor	Bad	Total
1.	2 min	0	0	1	9	10
2.	10 min	0	0	2	8	10
3.	30 min	0	0	2	8	10
4.	60 min	0	0	2	8	10
Total		0	0	7	33	40

With 5% solution, only one section showed excellent staining quality and three sections showed good quality; the best time duration for these results was 60 minutes (table 2).

Table (2) Show results of staining with 5% Hibiscus solution in different times

NO	Time	Excellent	Good	Poor	Bad	Total
1.	2 min	0	0	2	8	10
2.	10 min	0	0	3	7	10
3.	30 min	0	1	3	6	10
4.	60 min	1	2	3	4	10
Total		1	3	11	25	40

With 10% solution, only one section showed good staining quality in 60 minutes duration. No excellent results were noticed (table 3).

Table (3) Show results of staining with 10% Hibiscus solution in different times

NO	Time	Excellent	Good	Poor	Bad	Total
1.	2 min	0	0	1	9	10
2.	10 min	0	0	1	9	10
3.	30 min	0	0	3	7	10
4.	60 min	0	1	4	5	10
Total		0	1	9	30	40

With 100% staining solution, two sections showed good results in 60 minutes duration. Also, no excellent results were noticed (table 4).

Table (4) Show results of staining with 100% Hibiscus in different times

NO	Time	Very good	Good	Poor	Bad	Total
1.	2 min	0	0	1	9	10
2.	10 min	0	0	2	8	10
3.	30 min	0	0	4	6	10
4.	60 min	0	2	4	4	10
Total		0	2	11	27	40

Most of sections in the study (95.6%) showed poor or bad staining quality with Hibiscus solution.

In previous work of the authors, Hibiscus solution has given encouraging results with intestinal tissue [7] and renal tissue [8]. In this study, results seem to be in the opposite direction. It seems that squamous epithelial tissue (skin) is more resistant to penetration of Hibiscus solution than glandular tissues (renal and intestinal). Once there are excellent and good results, however, it seems only some modifications are needed to obtain larger numbers of better results.

Hashim EA [9] prepared 20% concentration of watery extracts of Hibiscus Sabdariffa and used it to stain renal tissue and vascular smooth muscle from albino mice to see the possibility of using these extracts as natural histological stain instead of eosin. The author reported that stained

tissues revealed acceptance to Hibiscus solution stain. That agrees with our previous studies on glandular tissues [7, 8].

CONCLUSION

As a conclusion from this study, Hibiscus Sabdariffa solution can be used as a cytoplasmic stain instead of eosin in the H&E method to stain formalin-fixed paraffin-embedded skin tissue sections. However, further studies with some modifications in temperature, duration of staining and PH are highly recommended to obtain better results.

ACKNOWLEDGEMENTS

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

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