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RESEARCH ARTICLE

STAINING OF HISTOLOGICAL SECTIONS FROM THE SMALL INTESTINE USING HIBISCUS SABDARIFFA

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ABSTRACT

Objective: this was a descriptive study aimed to assess the quality of staining of intestinal tissue by *Hibiscus Sabdariffa* solution compared to Hematoxylin-Eosin routine stain.

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

Results: With 5 % solution, 48 % of slides showed at least very good quality of staining. The excellent results were mainly obtained when time duration was 60 minutes while poorest results were obtained when staining time was 1-2 minutes

Conclusion: Hibiscus Sabdariffa solution is a cheap, natural, and harmless dye that can be used for staining of histological sections and it is comparable to the routine Hematoxylin and Eosin stain.

Key words: Hibiscus Sabdariffa; Histological Staining; Small Intestine

INTRODUCTION:

Routine Hematoxylin and Eosin (H&E) staining plays a critical role in tissue-based diagnosis. [1] By coloring tissue structures (cytoplasm, nucleus, organelles, and extra-cellular components), these stains allow the pathologist to view in details, under a microscope, tissue morphology and look for any abnormalities. [2] Even when advanced staining methods are used, the H&E stain still forms a critical part of the diagnostic picture as it displays the underlying tissue morphology which allows the pathologist to correctly interpret the advanced stain. [3]

Recently, natural dyes are promising to be cheaper potential sources for histological staining. [4] They are cheap, available, harmless, easier in application, and can substitute Hematoxylin or Eosin [5] *Hibiscus Sabdariffa* (Karkade) is an important one of these natural sources. It is a hardy herbaceous shrub belonging to the Family Malvaceae [6] and Sudan is the world largest producer and exporter of this plant (Karkade). [7]

The staining potential of *Hibiscus Sabdariffa* was poorly explored. The current study tried to explore *Hibiscus Sabdariffa* and assess its staining quality when applied on tissue sections from small intestine.

MATERIAL AND METHODS:

This was a descriptive study conducted during December 2013 at the histopathology laboratory of Portsudan Ahlia College. Archival formalin-fixed paraffin-embedded tissue blocks of small intestine were used in this study. Hundred-sixty 3-5 µ m-thick tissue sections were cut by a rotary microtome and divided into 4 equal groups (40 sections each group). Each group was stained by one of the following concentrations of the Hibiscus solution: 1%, 5%, 10%, or 100%, in different time durations (1-2 minutes, 10 minutes, 30 minutes, and 60 minutes) at room temperature.

Roselle staining solution was prepared by adding 1 g, 5 g, 10 g, or 100 g of *Hibiscus Sabdariffa* powder to 100 ml distilled water to obtain 1%, 5%, 10%, or 100% concentration respectively. Other slides stained by haematoxylin and Eosin were prepared as control. Stained slides were then assessed under a light microscope and the obtained data was analyzed by using SPSS software.

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RESULTS:

Quality of staining by Roselle was considered excellent when all the following were clearly seen under the light microscope: cell membrane, nuclear membrane, cytoplasm transparency, and extracellular matrix. If one of them was not obvious, the quality was considered very well. If two, it was good. If only one was obvious, quality was considered poor.

With 1 % solution, 5 % of slides showed excellent staining and 30 % showed very good quality of staining. Excellent results were mainly obtained when time duration was 30 or 60 minutes while poorest results were obtained when staining time was 1-2 minutes (Table No 1).

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

Time Duration	Excellent	V. Good	Good	Poor
1-2 min	0	2	1	7
10 min	0	3	1	6
30 min	1	3	1	5
60 min	1	4	1	4

With 5 % solution, 23 % of slides showed excellent staining and 25 % showed very good quality of staining. Excellent results were mainly obtained when time duration was 60 minutes while poorest results were obtained when staining time was 1-2 minutes (Table No 2).

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

Time Duration	Excellent	V. Good	Good	Poor
1-2 min	0	1	3	6
10 min	1	2	4	3
30 min	2	4	2	2
60 min	6	3	1	0

With 10 % solution, about 3 % of slides showed excellent staining and about 8 % showed very good staining. Excellent results were mainly obtained when time duration was 60 minutes while poorest results were obtained when staining time was 1-2 minutes. (Table No 3).

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

Time Duration	Excellent	V. Good	Good	Poor
1-2 min	0	0	2	8
10 min	0	0	3	7
30 min	0	1	4	5
60 min	1	2	3	4

With 100 % solution, about 15 % of slides showed excellent staining and about 13 % showed very good staining. Excellent results were mainly obtained when time duration was 60 minutes while poorest results were obtained when staining time was 1-2 minutes. (Table No 4).

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

Time Duration	Excellent	V. Good	Good	Poor
1-2 min	0	0	4	6
10 min	1	1	3	5
30 min	2	2	3	3
60 min	3	2	3	2

DISCUSSION:

To our knowledge, the current study represents the first initiative of using *Hibiscus Sabdariffa* extract in staining of intestinal histological sections in Sudan. The results are encouraging; almost half the stained sections (48%) showed excellent and very good staining quality. The only

disadvantage noticed was that most sections required 60 minutes to obtain better results. However, about 10 % of sections (15 sections) showed excellent and very good results within 30 minutes and about 5% of slides (8 sections) showed excellent and very good results within 10 minutes. Some local environmental factors, such as instability in electric supply, may explain the variability of results.

Hibiscus Sabdariffa has several nutritional and therapeutic benefits. [8, 9, and 10] Few researchers tried to apply modified extract solutions of the plant in diagnostic medical laboratory procedures.

Egbujo EC and colleagues [11] applied water extracts of Roselle with various modifications on a rabbit testicular tissue sections. They obtained best staining results when iron alum or potassium alum were used to mordant the extract.

Benard Solomon [12] stained formalin-fixed paraffin-embedded tissue sections using Hibiscus Sabdariffa extract mixed with ferric chloride and glacial acetic acid and reported efficiency of the solution as a progressive nuclear stain substitute for hem alum in H&E procedure.

Eman A. Hashim [13] reported that the purified acidic part of Hibiscus Sabdariffa could be used instead of eosin because this part has similar physical and chemical characteristics to the eosin stain.

Ihuma et al [14] applied methanolic extracts of H.sabdariffa to stain tissues with fungal infections and obtained diagnostic results.

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, careful adjustment of the surrounding environmental factors, especially temperature and pH, is needed in the coming research on use of Hibiscus *Sabdariffa* solution in histological staining of tissues.

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