



RESEARCH ARTICLE

STAINING OF HISTOLOGICAL SECTIONS FROM LIVER TISSUES USING CURCUMA LONGA

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ABSTRACT

Background and Objectives: natural plant dyes have gained worldwide interest and give encouraging tissue staining results when tested. This study aimed to assess the quality of staining of hepatic tissues by Hematoxylin and curcuma longa solutions compared to Hematoxylin and Eosin routine stain.

Methods: tissue biopsies were obtained from a liver of a healthy rabbit and stained using Curcuma Longa solution instead of eosin in the different Hematoxylin-eosin protocols.

Results: The best results were obtained with the time duration 10 – 20 min when using Weiger's Hematoxylin.

Interpretation and Conclusions: Curcuma longa extract is a promising histological dye that effectively can replace Eosin stain in the Hematoxylin & Eosin staining protocol.

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INTRODUCTION

The routine Hematoxylin and eosin staining protocol has been used for several decades and is still very important for identifying different tissue types and their physiological and/or pathological changes (Fischer, 2008), for many years, the stain has been unchanged because it gives good results with various fixatives and displays a wide range of cellular and extracellular details (Carleton, 1976 and Baker, 1979). Recently, the use of natural plant dyes has gained great interest. That may obviously be noticed in many developing countries because of the somewhat expensive prices and increasing costs of the synthetic dyes; therefore, the use of cheaper, naturally occurring plant dyes from is being an alternative to synthetic dyes (Evans, 1998; Siva, 2007). Curcuma longa is a perennial herb and a member of the Zingiberaceae (ginger) family, which proved benefit as an antioxidant, hepatoprotective, anti-inflammatory, anticarcinogenic and antimicrobial agent (Siva, 2007 and Ishita, 2004). The yellow dye of the Curcuma longa has been tested as a natural dye and found to have potential histological

application (Ishita, 2004). Curcuma longa has acidic reaction and strong affinity to the cytoplasm of cells and stain it yellow (Sachan, 2007 and Bhuyan, 2005). This shows that the reaction of the Curcuma longa stain is similar to the reaction of eosin in the Hematoxylin and Eosin technique except for its yellow coloration. For this reason and for its availability and cheap price in Sudan, authors were encouraged to plan for testing Curcuma longa as a counter stain and substitute for eosin in the Hematoxylin and Eosin routine technique.

MATERIAL AND METHODS

This analytical descriptive study included tissues from the liver of a healthy rabbit, fixed in 10% formalin, embedded in paraffin, cut by a rotary microtome into 3-5 µm-thickness sections, and then made into three equal groups; the first group was stained using Mayer's Hematoxylin and Curcuma Longa, the second group was stained by Harri's Hematoxylin and Curcuma Longa, and the third group was stained by Weiger's Hematoxylin and Curcuma Longa. Each group was subdivided into four subgroups; the first subgroup was stained for 1 to 5 minutes, the second for 10 to 20 minutes, the third for 25 to 40 minutes, and the fourth subgroup for 50 to 60 minutes. Total number of sections was 120. Water extracts of Curcuma Longa

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without additions were used instead of Eosin Solution in Hematoxylin staining protocol (Harri's, Meyer's and Weiger's) to stain cytoplasm and extracellular elements. The study was carried out at the histopathology laboratory of Port Sudan Ahlia College, Portsudan city, Red Sea State, Sudan, during the period between January and July 2017.

RESULTS

Quality of staining by Curcuma Longa is considered excellent when all the following four structure parts are clearly seen and identified under the light microscope: cell membrane, nuclear membrane, cytoplasm transparency, and extracellular matrix. If one of them is not obviously seen, the quality is considered good. If two, it is considered accepted. If only one structure is obvious, quality is considered poor. Staining by Mayer's Hematoxylin showed poor results in most sections (62.5%). The best results were obtained with the time duration 10 – 20 min in only one section. That is shown in table number 1.

Table 1. Staining of the liver tissue by Mayer's Hematoxylin and Curcuma Longa

Time Duration	Excellent	Good	Accepted	Poor	Total
1-5 min	0	0	3	7	10
10-20 min	0	1	8	1	10
25 – 40 min	0	0	3	7	10
50 – 60 min	0	0	0	10	10
Total	0	1	14	25	40

Staining by Harri's Hematoxylin showed good results in only three sections (about 8%) with the best results obtained in 10-20 min duration. That is shown in table number 2.

Table 2. Staining of the liver tissue by Harri's Hematoxylin and Curcuma Longa

Time Duration	Excellent	Good	Accepted	poor	Total
1 – 5 min	0	1	8	1	10
10 – 20 min	0	2	8	0	10
25 – 40 min	0	1	9	0	10
50 – 60 min	0	0	7	3	10
Total	0	4	32	4	40

Staining by Weiger's Hematoxylin showed good results in 15 sections (about 38 %) with the best results obtained with time durations 1- 5 min and 10 – 20 min. That is shown in table number 3.

Table 3. Staining of the liver tissue by Weiger's Hematoxylin and Curcuma Longa

Time Duration	Excellent	Good	Accepted	Poor	Total
1 – 5 min	0	8	2	0	10
10 – 20 min	0	7	3	0	10
25 – 40 min	0	0	6	4	10
50 – 60 min	0	0	3	7	10
Total	0	15	14	11	40

Unfortunately, no excellent results were noticed in this study.

DISCUSSION

The use of natural dyes has been increased worldwide to decrease cost and minimize hazardous during laboratory work. The present study is one in the track of exploring laboratory properties of extracts from medical plants, here it is Curcuma Longa. Curcuma longa extract is a promising histological dye that is not only cheap but readily available. It could serve as a useful alternative to eosin in developing countries. In this

study, the best results were obtained when using Curcuma longa extract with Weiger's Hematoxylin in a time duration of 10 – 20 min. Some other studies agree with the findings in this study. Kumar S et al (Sachin Kumar, 2014), reported that Curcuma longa extract can be used as a counterstain after Hematoxylin, giving good staining ability and comparable to that of eosin dye. O. G. Avwioro et al (Avwioro, 2007), studied Human tissue samples obtained from the skin, liver, intestine, kidney, lung and spleen at post-mortem examination and found that the reaction of the C. longa stain is similar to that of eosin in the Hematoxylin and eosin technique except for its yellow coloration. Therefore, for its high pigment content it was used as a substitute for eosin in the Hematoxylin and eosin technique. As a conclusion from this study, Curcuma Longa has proved to be efficient natural dye in staining of histological sections from the liver and it is comparable to the routine Hematoxylin and Eosin stain. Curcuma longa staining procedure does not need high qualified personnel so it can replace eosin in routine procedure. Authors strongly recommend using of Curcuma longa in histopathology & cytology laboratory because of availability and cheap price, in addition to its easy preparation. However, some modifications in concentration and/or time duration may be needed to increase accuracy of the technique.

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