International Journal of Health Research and Innovation, vol.4. no.1, 2016, 31-38
ISSN: 2051-5057 (print version), 2051-5065 (online)
Scienpress Ltd, 2016

Hibiscus-Shorgum: A New Morphological Stain in Neuro-Histology

Muhammed Abdurrasheed Ola[1], Olutunde Oluwumi Abiola[2], Benard Solomon Adebayo[2], Muhammad Ahmad Tangaza[1] and Omoowo Balikis Tanwa[2]

Abstract

Aim: The objective of this study was to investigate the capability of the Hibiscus & Sorghum extracts to replace Haematoxylin & Eosin stains in the brain cells demonstration.

Methods: Tissue blocks of Wistar rat brain were retrieved from the Animal Tissue Block Archive of the Department of Pathology, University of Ilorin Teaching Hospital Ilorin.

Four normal blocks were randomly selected and Four (4) serial sections labeled A to D were made from each block and stained as follows; **A**: Haematoxylin and Eosin (H&E), **B**: Hibiscus and Eosin (H-E), **C**: Haematoxylin and Sorghum (H&S), and **D**: Hibiscus and Sorghum (H-S).

Results: The photomicrographs from all the groups presented the hippocampal cells in the same and almost indistinguishable manner. The layers, neurones and the glial cells were well stained. However, very distinct colorations of the hippocampal components comparable to the control, was noted in H-S (the group D).

Conclusion: This study established the capability of the H-S to replace H&E in the neuro-histoarchitectural studies. This substitution is necessary because of the domestic availability, ease of preparation and excellent neural cells demonstration

[1] Department of Histopathology, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Sokoto State, Nigeria.
[2] Histopathology Unit, Department of Pathology, University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria

by Hibiscus and Sorghum stain extracts.

Keywords: Hibiscus, Sorghum, Hippocampus, Glial cells

1 Introduction

Hematoxylin and Eosin (H&E) stains play a critical role in tissue-based diagnosis [1].The two, are the commonest stains being used in histopathology laboratories all over the globe which colour the nuclei a dark blue or purple, and the cytoplasm and connective tissue a shades of pink [2]. By colouring tissue structures (cytoplasm, nucleus, organelles, and extra-cellular components); these stains allow the sections to be viewed in details under the microscope, tissue morphology and/or any present abnormalities can then be easily detected [3]. 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 correct interpretation to be made [4]. Most histological stains in current use are of synthetic origin; however, natural dyes are still promising to be cheaper potential sources [5]. Any development of new histological stain is justified if the new stain is cheaper, available, harmless, and easier in application [6].
Hibiscus sabdariffa belongs to the vascular flowering plants, known as Roselle or Red Sorrel in English and Karkade in Arabic [7]. It is a hardy herbaceous shrub belonging to the Family Malvaceae [8]. 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 [8].

Sorghum bicolor originated from North-Eastern tropical Africa (possibly Ethiopia) domesticated from as early as 5,000- 3,000BC to around 1000BC. From North-Eastern Africa sorghum was distributed all over Africa, through the Middle East to India. From India to China, from West Africa sorghum was taken to America through slave trade [9]. It is typically an annual, but some cultivars are perennial. It grows in clumps that may reach over 4 meters [9].*Sorghum bicolor* has a red dye present in the leaf sheath and sometimes in adjacent stem parts. In Africa, the dye is used for goat-skin leather (Nigeria) in local weaving design (Sudan) [10]. In some West African countries, the dye can be extracted from the plant to colour leathers, cloths, calabashes and as body pigment [11].
The hippocampus is anatomically one of the oldest regions of the brain. It is made up of interconnected layers of the dentate gyrus and cornuammonis. Each of the layers and/or subfields of the hippocampus have been shown to be involved in different hippocampal functions and tasks [12].
The extraction and application of colouring matters from *Hibiscus sabdariffa*and

Sorghum bicolor will be of great contribution to the exploitation of natural dyes and their applications, most especially in the field of neuropathology. This research wants to contribute to the use of natural dyesfrom *H. sabdariffa* and *Sorghum bicolor* as a substitute for haematoxylin and eosin in the staining of human and animal brain tissues in the field of histopathology.

3 Methodology

Sorghum bicolor stalks and *Hibiscus sabdariffa* (Dry leaves) were purchased in a local market in Ilorin, and were identified by a Botanist in the Botany department of Obafemi Awolowo University, Ile-Ife, Nigeria. They were processed using the techniques of Omoowo and colleagues [13] and Benard [14] respectively. 10% Formalin fixed, paraffin wax processed brain tissues were sectioned at 3 microns. Slides of serial sections produced were tagged and stained as follows: A: Haematoxylin and Eosin (H&E), B: Hibiscus and Eosin (H-E), C: Haematoxylin and Sorghum (H&S), and D: Hibiscus and Sorghum (H-S).

1. PREPARATION OF STAIN EXTRACTS

1.1 HIBISCUS EXTRACT SOLUTION

The dry calyces of *Hibiscus sabdariffa*were ground using a Binatone blender to a fairly powdery form. To 10g of the ground red calyces of *H. sabdariffa* in a conical flask, 200ml of distilled water was added and brought to boil to give the brilliant red coloured extract which was immediately allowed to cool and filtered to give a clear *H. sabdariffa* extract. The staining formula was compounded as follows:
H. sabdariffa extract 100ml

NaCl 5.0g
10% ferric chloride solution 1.2ml
Glacial acetic acid 3.0ml

1.2 10% ALCOHOLIC EXTRACT OF SORGHUM

S. bicolor stalk was ground into a powdery form with a Binatone blender. 10g of the ground powder was weighed using a sensitive balance (Ohaus) and dissolved in a conical flask containing 100ml of absolute alcohol. The solution was allowed to stay for 24 hr at room temperature (25±2ºC) after which it was filtered into a staining jar.

2. STAINING PROCEDURES

2.1 H&E

- Section was dewaxed in xylene and hydrated through 100%, 90%, 70%, 50% alcohol to water
- It was stained in Harris haematoxylin for 15 minutes
- Rinsed in water
- Differentiated in 1% acid alcohol
- Blued in running tap water for 10 minutes
- Counter-stained in 1% alcoholic eosin for 1 minute
- Dehydrated in ascending grades of alcohol
- Cleared in xylene and
- Mounted with DPX

2.2 H-E

- Sections were taken to water through 100%, 90%, 70%, 50% alcohol and water
- Stained in Hibiscus extract solution for 5 minutes
- Washed in running tap water for 2 minutes
- Counter-stained in 1% alcoholic Eosin for 1 minute
- Dehydrated in ascending grades of alcohol
- Cleared in xylene and
- Mounted with DPX

2.3 H&S

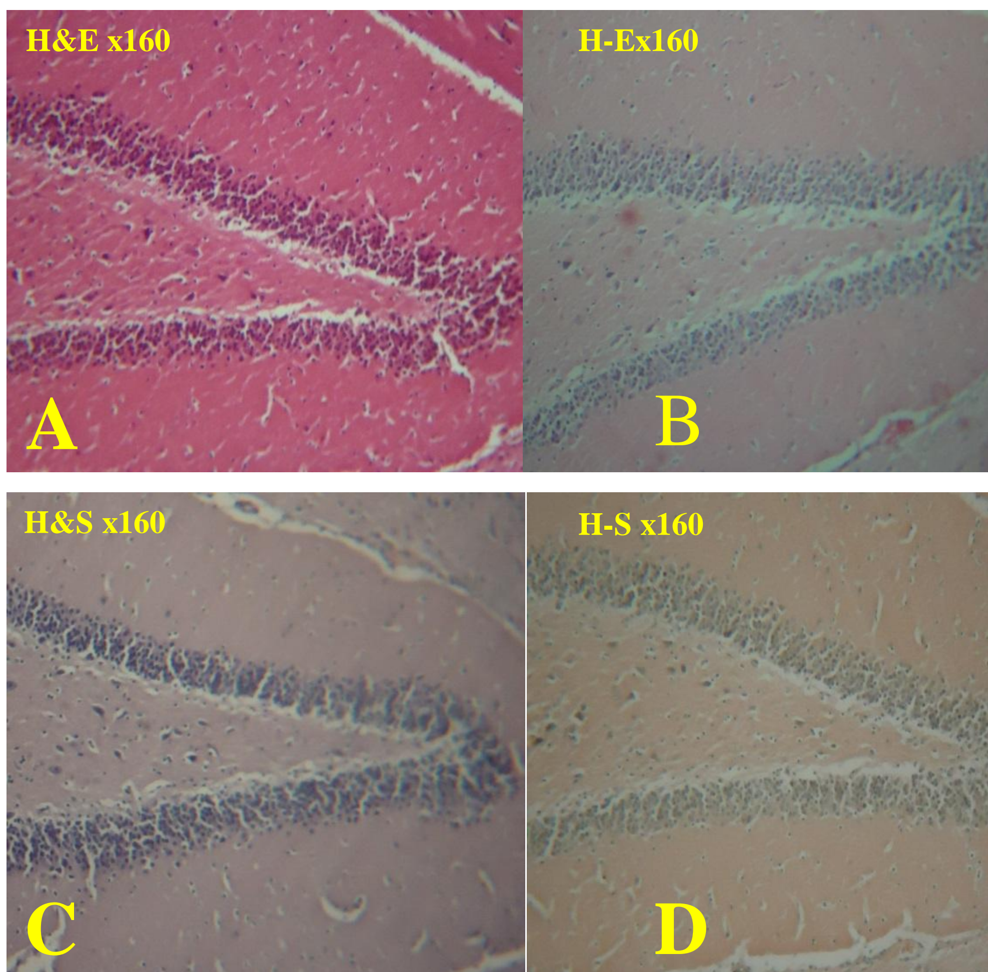
- Section was dewaxed in xylene and hydrated through 100%, 90%, 70%, 50% alcohol to water
- It was stained in Harris haematoxylin for 15 minutes
- Rinsed in water
- Differentiated in 1% acid alcohol
- Blued in running tap water for 10 minutes
- Counter-stained in 10% alcoholic Sorghum for 3 minutes
- Dehydrated in ascending grades of alcohol
- Cleared in xylene and
- Mounted with DPX

2.4 H-S

- Sections were taken to water through 100%, 90%, 70%, 50% alcohol and water

- Stained in Hibiscus extract solution for 5 minutes
- Washed in running tap water for 2 minutes
- Counter-stained in 10% alcoholic Sorghum for 3 minutes
- Dehydrated in ascending grades of alcohol
- Cleared in xylene and
- Mounted with DPX

4 Results



The photomicrographs of the sections of group A-D are shown above as designated: The histomorphological features of the Hippocampal layers, the neurones as well as the glial cells were distinctly preserved across all the groups. The H-E stained section (group D), had the closest resemblance to the H&E (The positive control group).

5 Conclusion

Before now, interest of the scientists in histopathology has not been directed towards finding alternatives to the age long Haematoxylin and Eosin stains; despite its cost and scarce availability. The few available literatures on the topic seem very recent.
The nuclear staining capability of *Hibiscus sabdariffa* has been established by Benard [14]. He 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 along with parallel Haematoxylin 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. He then suggested that the extract solution could be a progressive nuclear stain substitute for hemalum in H&E procedure.
Likewise, Omoowo et al [13] established the counter-staining potentials of stalk extracts of *S. bicolor*, and specifically recommended the absolute ethanolic extract of 10% *S. bicolor* which has the closest morphologic resemblance, as a substitute for eosin in diagnostic histopathology staining.
This study had tested the applicability of the established techniques of Benard [14] and Omoowo et al [13] on the brain tissue. Hippocampus was specifically selected due to its dense population of neurons, glial cells and distinct interconnected layers of the dentate gyrus and cornuammonis [12]. Sections from Hibiscus-Sorghum (group D) showed the neural cells nuclei with brilliant blue-black colouration, and the cytoplasm stained pinkish brown. This was the same reports from the previous authors [14][13] though; none of these authors had earlier worked on brain tissue.
Eman et al[15] 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. Likewise, Egbujo et al [16] prepared Roselle (*Hibiscus sabdariffa*) aqueous extracts with various modifications, and used it for the differential staining of rabbit testicular tissue sections. He reported various levels of differentiation of nuclear and cytoplasmic structures as well as other structures of this organ with 1% eosin as a counter stain. The best staining result was reportedly obtained when iron alum was used to mordant the extract and when the extract mordanted with potassium alum was acidified using acetic acid.
Methanolicextract of *H. sabdariffa* has been used as a staining agent for some fungi and therefore hope to reduce the problems associated with over-dependence on toxic, expensive and scarcely available exotic stains [17].
Ibnouf and colleagues had also in two different publications, confirmed the staining ability of aqueous extract of *H. sabdariffa,* and suggested that it may be good as a replacement for eosin; though with the shortcoming of too long staining period [18][19].

The good results recorded from the exchange and substitution of one stain for the other (as demonstrated in group B and C) testified to the flexibility of the new H-S technique, and equally attested to the iso-electrical compatibility of the four stains [20].

H. sabdariffa, according to Egbujo et al [16] and Benard [14] can be substituted for haematoxylin in H&E procedure. However, Ihuma [17], Ejugbo et al [16], Eman et al[15] and Ibnouf et al [18] reported its counter-staining characteristics, and recommended its replacement for eosin. Because of these variance reports, new research is underway to investigate the possibility of using only the extracts of this plant as both nuclear and cytoplasmic stains at different manipulation.

Conclusively, this new staining technique (H-S) could be a substitute to H&E staining technique in neurological studies because the neural components were clearly demonstrated. It could therefore be a useful tool in diagnostic neuropathology.

References

[1] J. A Kiernan, Histological and Histochemical Methods: Theory and Practice. 4th ed, Bloxham, UK: Scion, 2008.

[2] Cellpath, Staining theory. 2006,67-72

[3] S. Iyiola and O. G Avwioro,"Alum haematoxylin stain for the demonstration of nuclear and extra nuclear substances". Journal of Pharmacy and Clinical Sciences, 1, (2011),20-23.

[4] O. GAvwioro,"Histochemical Uses of Haematoxylin - A Review". Journal of Pharmacy and Clinical Sciences,1, (2011), 24-34.

[5] H. I Mattuk, Studies on the utilization of natural colorants extracted from some plant sources. Egypt J. Appl.Sci., 13, (1998), 286-303.

[6] D. P Penney, J. M Powers,M. Frank and C.Churukian, Analysis and testing of Biological Stains–the Biological Stain Commission Procedures. Biotechnic& Histochemistry, 77, (2002), 237-275.

[7] B. H Ali, N. Al-Wabel and G. Blunden, Phytochemical, pharmacological and toxicological aspects of a Hibiscus Sabdariffa; a review. Phytotherapy Research, 19(5), (2005), 369- 375.

[8] Y. Qi, K. L Chin, F. Malekian, M. Berhame, and J. Gager, Biological characteristics, nutritional and medicinal value of Roselle Hibiscus Sabdariffa. Circular-Urban Forestry Natural Resources and Environment,604, (2005), 1-2.

[9] A. Umar, Effect of Raw and Modified Sorghum Cellulose Fibre on the Mechanical Properties of Natural Rubber Composite. MSc. Dissertation, Dept. of Pure and Industrial Chemistry, BayeroUniversity Kano, 2009.

[10] T. V Balole and G. M Legwaila, Sorghum bicolor (L.)Moench (Internet) record from protabase. Jansen. P.C.M and Cardon D. (Editors) 2008.

[11] L. S Cobleyand W. M Steele, An introduction to the botany of tropical crops. 2nd ed, 1976, 43-45.

[12] S. Leutgeb,J. K Leutgeb,A. Treves, M.B Moser andE.I Moser, Distinct ensemble codes in hippocampal areas CA3 and CA1. Science, 305, (2004), 1295-1298

[13] B. T Omoowo, J. K Bankole, A. O Muhammed, S. A Benard and O. O Afolabi, Histomorphological assessment of the extract of the stalk of Sorghum bicolor as counter stain. African Journal of Cellular Pathology,2, (2014), 69-74.

[14] S. A Benard, Iron-Roselle: A Progressive nuclear stain substitute for hematoxylin. The Journal of Histotechnology, 31(2), (2008), 57-59.

[15] A. H Eman, The use of watery extract of Kujarat flowers Hibiscus Sabdariffaas a natural histological stain. Iraqi Journal of Med Sci,5(1), (2006), 29-33.

[16] E. C Egbujo, O. J Adisa and A. B Yahaya, A Study of the Staining Effect of Roselle (Hibiscus Sabdariffa) on the Histologic Section of the Testis. International Journal of Morphology,26(4), (2008), 927-930.

[17] J. O Ihuma, G. H Asenge, J. O. K Abioye, and S. K Dick, Application of Methanolicextracts from Hibiscus Sabdariffa Linn as a biological staining agent for some fungal species. International Journal of Plant, Animal and Environmental Sciences, 2(2), (2012), 254-259.

[18] A. Ibnouf, E. AbdulRaheem, M. SeedAhmed and D. Dahab, Assessment of Staining Quality of Roselle (Hibiscus sabdariffa) on Formalin Fixed Paraffin-Embedded Renal Tissue Sections. IJCRR, 6(21), (2014), 26-28.

[19] A. Ibnouf, K. Adam, E. AbdulRaheem and A. Ageep, Staining of Histological Sections from the Small Intestine using Hibiscus sabdariffa. Journal of Biomedical and Pharmaceutical Research,3(5), (2014), 01-03.

[20] S. ABenard, A. OMuhammed, A. A Fowotade, O.O Afolabi andO.A Olutunde,Sorghum bicolor Extract: A Suitable Counter Stain in Hibiscus Extract Nuclear Staining of Liver and Kidney.African Journal of Cellular Pathology,4, (2015),13-16.