

HIBISCUS EXTRACT-IRON-VAN GIESON: A NEW MORPHOLOGICAL STAINING TECHNIQUE IN NEURO-HISTOLOGY

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ABSTRACT

Aim: To investigate the suitability of Hibiscus extract mordanted with ferric chloride and counterstained with Van Gieson in the demonstration of brain histomorphology.

Method: 10% formol saline fixed brain tissues from cerebellum, cerebrum and basal ganglia were processed by the paraffin wax technique, sectioned and stained with H&E labeled A, Hibiscus/Van Gieson stain labeled B and Weigert's Van Gieson stain labeled C as control.

Results: Photomicrographs from all the staining techniques presented all the brain cells especially the molecular cell layer and the granular cell layer of the cerebellum in comparable manner. The cells were stained blue-black with the Hibiscus-Van Gieson (B) and Haematoxylin van Gieson (C) methods.

Conclusion: The suitability of Hibiscus extract-Iron-van Gieson in the histomorphological demonstration of brain tissue is established.

Key words: Hibiscus stain, Van Gieson, neuro-histology, roselle.

INTRODUCTION

Weigert's iron haematoxylin is used for the staining of cell nuclei when subsequent staining reagents contain acid such as in van Gieson stain which will decolorize nuclear staining if stained previously with haematoxylin mordanted with potassium or ammonium alum. The Weigert's haematoxylin is stored separately from the mordant (acidified ferric chloride). Equal volumes are mixed immediately before use (Avwioro, 2011). The resulting colour should be purple- black with a staining time of 20-30 minutes. Weigert's iron haematoxylin is used for the staining of cell nuclei when demonstrating collagen and muscle with the van Gieson stain and the trichrome connective tissue stains (Avwioro, 2002). Van Gieson's Stain is a counter stain that is commonly used for the demonstration of collagen. It was named after American bacteriologist Ira Van and comprises two acid dyes- picric acid and acid fuchsin (Nicola, 2014). Van Gieson as a special stain was earlier reported to stain well with anthocyanin from black berry when he used it as

a counter stain (Al-Tikriti & Walker, 1977). Anthocyanin has also been found to be the staining pigment in Hibiscus sabdariffa (Benard, 2008). Hematoxylin and Eosin (H&E) stains play a critical role in tissue-based diagnosis (Kiernan, 2008). Haematoxylin was first used successfully as a nuclear stain in histological procedures in 1863. It became a universal staining method when it was combined with eosin in 1875 by Wissowzky (Benard, 2008). 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 (Iyiola and Avwioro, 2011). Although haematoxylin and eosin is the most useful general tissue staining method, it fails however to demonstrate many of the specific brain cells and their processes. For this reason, some neuropathologists prefer to use Weigert's haematoxylin and van Gieson in place of the H&E stain. (Lynch, 1976). The plant Hibiscus

sabdariffa is very popular among the peoples of Northern Ghana, where the leaves are used in soups and calyces for soft drinks and also used medicinally. It has been found to possess several health benefits (Dokosi, 1998). The aqueous extract of the dry red calyx is often prepared as a drink for refreshment locally called 'zobo' in Nigeria (Adegunloye et al., 1996). Vernacular names, in addition to roselle, in English speaking regions are rozelle, sorrel, red sorrel, Jamaica sorrel, Inidan sorrel, Guinea sorresl, sour-sour, Queensland jelly plant, jelly okra, lemon bush, and Florida cranberry. In French, roeselle is called oseille rouge, or oseille de Guinée; in Spanish, quimbobobó chino, serene, rosa Jamaica, flor de Jamaica, Jamaica, argria, agrio de Guinea, quetmia ácida, vina and viñuela; in Portuguese, vinagreira, azeda de Guiné, cururú azédo, and quiabeiro azédo; in Dutch (Surinam), zuring. In North East, roselle is called karkadé or carcadé, and it is known by these names in the pharmaceutical and food-flavoring trades in Europe. In Senegal, the common name is bisap. The plant is widely grown in Nigeria and other sub-Saharan African countries as a crop used for demarcation of farm plots (Benard, 2008). Hibiscus (roselle) also produces a brilliant red colour rich in anthocyanin, ascorbic acid and hibiscus acid (El-Nazar et al., 1991). Anthocyanins belong to a large group of plant pigments second only to chlorophyll. They appear in plants in the form of heterosides. The most likely candidates for the colorant in hibiscus include dephidin, petunidin, and malvidin. The core chromophore in anthocyanins is the flavylium nucleus (Cardon, 2007, Benard, 2008). Most histological stains in current use are of synthetic origin; however, natural dyes are still promising to be cheaper potential sources (Mattuk, 1998). Any development of new histological stain is justified if the new stain is cheaper, available, harmless, and easier in application (Penney et al., 2002). The extraction and application of colouring matters from Hibiscus sabdariffa will be of further contribution to the exploration of local natural dyes and their applications, most especially in the field of neuro-histology. This research work is an attempt to contribute to the use of local natural dye from H. sabdariffa as a suitable stain for human and animal brain tissues in the field of histopathology.

MATERIALS AND METHODS

Hibiscus sabdariffa dry leaves were purchased in a local market in Ilorin, and identified by a Botanist in the department of Obafemi Awolowo University, Ile-Ife, Nigeria. They were processed using the technique of Benard (2008). 10% formalin fixed, paraffin wax embedded brain tissues were sectioned at 4 microns and slides of serial sections produced were labeled A: Haematoxylin & Eosin, B: Hibiscus-van Gieson and C: Weigert's -van Gieson. Mounted slides were examined microscopically and photomicrograph subsequently taken.

Preparation of Hibiscus Solution

The dry calyces of H. 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 distilled water was added and brought to boil to give the brilliant red colored extract which was immediately allowed to cool and filtered to give a clear H. sabdariffa extract. To compound the staining formular, 100ml of clear H.sabdariffa extract was mixed with 2g NaCl, 1.2ml of 10% ferric chloride solution and 3ml of glacial acetic acid.

Staining Method for Hibiscus/Van Gieson

Sections were dewaxed in xylene and hydrated through 100%, 90%, 70%, 50% alcohol to water and subsequently stained in Hibiscus extract solution for 5 minutes, washed in running tap water for 10 minutes, counterstained in Van Gieson for 3 minutes, dehydrated in ascending grades of alcohol, cleared in xylene and finally mounted in DPX.

Weigert's Haematoxylin/Van Gieson Staining Method

Sections were dewaxed in xylene and hydrated through 100%, 90%, 70%, 50% alcohol to water. Subsequently, sections were stained with 1 volume of Weigert's haematoxylin solution A (1% w/v haematoxylin in 95% ethanol) mixed with 1 volume of Weigert's solution B (95ml d/w, 4ml of 29% (aq) FeCl₃, and 1ml of conc HCl) for 20 minutes, rinsed in running tap water, differentiated in 1% acid alcohol, washed in running tap water for 10 minutes, counterstained in Van Gieson for 3 minutes, dehydrated in ascending grades of alcohol, cleared in xylene and mounted in DPX.

H & E Staining Procedure

Sections were dewaxed in xylene and hydrated through 100%, 90%, 70%, 50% alcohol to water and subsequently stained in Harris haematoxylin for 15 minutes, washed in running tap for 2 minutes, differentiated in 1% acid alcohol, washed in running tap water for 10 minutes, counter stained in 1% alcoholic eosin for 30 seconds and mounted in DPX.

RESULTS

Results show satisfactory staining of brain cells with the Hibiscus/van Gieson technique comparable to Weigert's haematoxylin/van Gieson. Particular interest is centered on the nucleus of the molecular and granular cells of the cerebellum which stains blue-black both for the Hibiscus solution and Weigert's haematoxylin.

DISCUSSION

Haematoxylin is most useful as a general routine method. It fails, however to demonstrate many of the specific cells and their processes, hence some neuropathologists prefer to use Weigert's haematoxylin and Van Gieson in place of the H and E stain. (Lynch, 1976). For years, interest of the scientists in histopathology has not been directed towards finding alternatives to the age long Haematoxylin despite its cost and scarce availability especially in low resource countries. The few available literatures on the topic seem very recent. According to earlier findings, Hibiscus extract which contain anthocyanins has been used as nuclear stain (Benard, 2008, Egbujo et al., 2008) and cytoplasmic stain (Abd-Alhafeez Ibnouf et al., 2014). It has also been counter stained with 10% Sorghum bicolor alcoholic extract as iso-electrically compatible stains (Benard et al., 2015). The suitability of Hibiscus counterstained with Sorghum bicolor extract as a neuro-histological stain to replace H&E has also been established as reported by Muhammed et al., (2016) after a previous description of its staining ability by Avwioro et al., (2006). Hibiscus extract mordanted with iron salts have been used to stain nuclear components as haematoxylin substitute with satisfactory results (Benard, 2008; Egbujo et al., 2008). This was however limited to appendix, lymph node, and testis. In a recent publication, Hibiscus extract nuclear staining was applied successfully on brain tissues (Benard et al., 2015). It was recommended that Hibiscus

extract could replace haematoxylin in H&E technique. Muhammed and colleagues in their study also used H. sabdariffa extract to stain nuclear components of the hippocampus with alcoholic extract of Sorghum bicolor as counter stain (Muhammed et al., 2016). It was recommended that Hibiscus-Sorghum being locally available and bio-friendly stains could replace H&E in the demonstration of brain cells of the hippocampus of Wistar rat. However, no work to the knowledge of the authors has combined H. sabdariffa with van Gieson to demonstrate brain morphology. Findings in this work reveal comparable and satisfactory staining of neuronal cell's nucleus and cytoplasmic content by the Hibiscus extract-iron-van Gieson technique and haematoxylin-van Gieson stain (See figure B and C). This work has by so doing, tested successfully the applicability of haematoxylin-van Gieson on brain morphology as recommended by Lynch (1976). The new staining technique stains nucleus blue-black, red blood cells yellowish-brown and cytoplasmic content brownish-red. The cellular presentation of the granular cells of the cerebellum is particularly impressive. The comparatively similar results obtained from the haematoxylin-van Gieson and Hibiscus-van Gieson methods infer that Hibiscus extract-iron-van Gieson can be successfully applied in the demonstration of brain cells. Details of the mechanism of staining need to be further investigated but the blue-black staining of the nucleus could be due to the anthocyanin-iron dye lake complex. Similar colour presentation has been reported in haematoxylin-iron dye lake complex (Lynch, 1976). This is a further confirmation of the comparable staining of H.sabdariffa extract with haematoxylin techniques as reported by earlier findings (Benard, 2008; Egbujo et al., 2008, Benard et al., 2015, Muhammed et al., 2016). The local availability of hibiscus extract in the new technique and its affordability in a low resource setting makes it a choice alternative to the haematoxylin stain. Interestingly too, its ability to resist fading over a long period of time is particularly encouraging. The authors highly recommend the new technique as a morphological stain in neuropathological studies.

CONCLUSION

Hibiscus Extract- Iron-Van Gieson staining technique shows promise as a suitable

morphological stain for brain tissue in the field of neuro-histology.

ACKNOWLEDGEMENT

We highly appreciate the support of Mr. Fowotade A.A., Chief Medical Laboratory Scientist, Pathology Department, University of Ilorin Teaching Hospital towards the success of this work.

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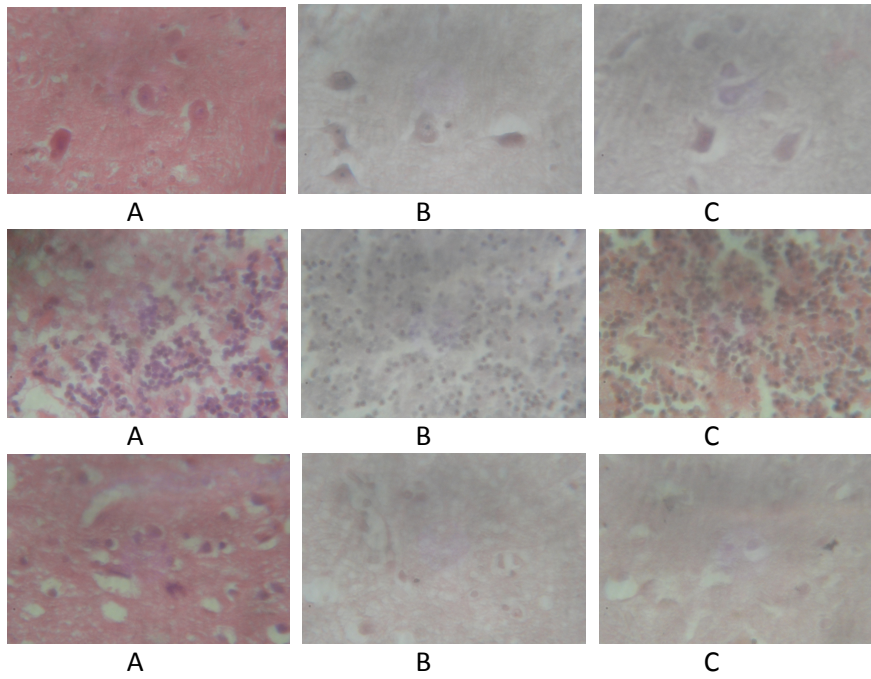
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A: H&E staining of brain showing granular cells & neuronal cell bodies:
B: Hibiscus- Van Gieson stain of brain morphology-granular cells & neuronal cell bodies. C: Weigert's Van Gieson Stain of brain morphology-granular cells & neuronal cell bodies. Mag. X 400