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HIBISCUS-VAN GIESON STAIN FOR COLLAGEN FIBRES

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Abstract

Aim: This is an experimental study aimed at exploring the capability of Hibiscus extract-iron solution counterstained with van Gieson stain to demonstrate collagen fibres in skin tissue.

Methods: 10% neutral buffered formalin fixed, paraffin embedded tissue blocks of skin were retrieved from the tissue block archive of the Department of Pathology, Unilorin Teaching Hospital, Ilorin, Kwara State, Nigeria. Two blocks were randomly selected and sectioned serially at 4 microns. The sections were primarily stained progressively with locally sourced and compounded Hibiscus sabdariffa extract-iron- solution. They were subsequently counterstained with van Gieson. Parallel sections were stained with HVG and H&E as controls.

Result: Photomicrographs reveal satisfactory staining of collagen fibers comparable to routinely used HVG.

Conclusion: This study established the capability of Hibiscus-van Gieson to demonstrate collagen fibres and could be useful in dermato-histoarchitectural studies.

Keywords: Hibiscus roselle, stain, van Gieson, skin, collagen

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INTRODUCTION

The plant Hibiscus sabdariffais 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).The plant is widely grown in Nigeria and other sub-Saharan African countries as a crop used for demarcation of farm plots (Benard, 2008). Different names have been ascribed to it depending on the language and locality (Benard et al., 2016). Hibiscus (roselle) also produces a brilliant red colour rich in anthocyanin, ascorbic acid and hibiscus acid (El-Nazar et al., 1991). 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 fuschin (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 and Walker, 1977). This was tested successfully when Hibiscus sabdariffa anthocyanin combined well with van Gieson to demonstrate brain morphology (Benard et al., 2016). There is paucity of research publications

on the use of Hibiscus nuclear stain with van Gieson counterstain. Earlier success with brain morphology informs this systematic study in order to contribute to knowledge on the application of Hibiscus-van Gieson staining technique in the demonstration of collagen fibres.. 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 dermatology.

Materials and Methods

Hibiscus sabdariffa dry leaves were purchased at 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% neutral buffered formalin fixed, paraffin wax embedded skin tissues from the block archive of the Pathology Department, University of Ilorin Teaching Hospital were retrieved and sectioned at 4 microns and slides of serial sections produced were labeled A: H&E, B: HVG and C: Hib-VG. Mounted slides were examined microscopically and were photomicrographs 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 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 mounted in DPX.

Weigert'sHaematoxylin/Van Gieson 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) $FeCl_3$, 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 water for 2 minutes, differentiated in 1% acid alcohol, washed and blued in running tap water for 10 minutes, counter stained in 1% alcoholic eosin for 30 seconds and mounted in DPX.

RESULTS

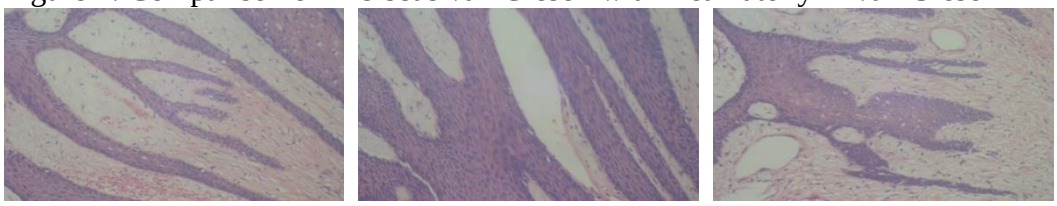
Results show satisfactory staining of skin morphology and collagen fibres with the Hibiscus-van Gieson technique comparable to Weigert's haematoxylin-van Gieson (HVG). Collagen stains red in the new technique as with standard HVG.

DISCUSSION

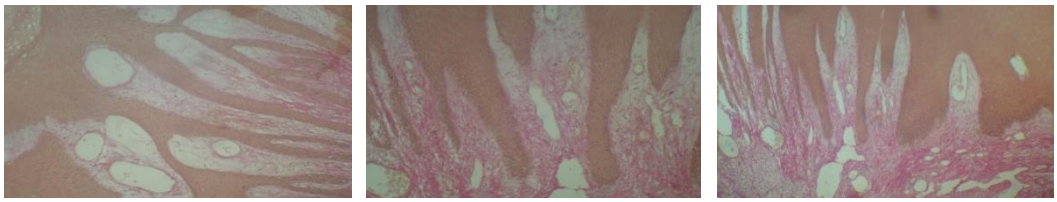
For years, interest of researchers in histopathology has not been directed towards finding alternatives to the age long Haematoxylin despite its cost and scarce availability especially in low resource countries. The few available literatures on the topic seem very recent (Muhammed et al., 2016; Benard et al., 2016). According to earlier findings, Hibiscus extract which contain anthocyanins has been used as nuclear stain (Benard, 2008, Egbujo et al., 2008) and cytoplasmic stain (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 and colleagues (2016). 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, liver and kidney. 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 extact of Sorghum bicolor as counter stain (Muhammed et al., 2016). It was recommended

that Hibiscus-Sorghum being locally available and bio-friendly could replace H&E in the demonstration of brain cells of the hippocampus of Wistar rat. In another recent publication, Hibiscus nuclear staining was combined electrostatically with van Gieson to demonstrate brain morphology, cerebellum, pons and cerebrum especially the neuronal cells and granular cells (Benard et al., 2016). They recommended Hibiscus-van Gieson as a new technique for the demonstration of brain cells. However, there is paucity of published work on the application of Hibiscus-van Gieson technique on connective tissue of skin especially collagen fibers. Findings in this work reveal comparable and satisfactory staining of collagen fibers by the Hibiscus extract-iron-van Gieson technique and haematoxylin-van Gieson stain {HVG} (See figure B and C). This work has by so doing, tested successfully the applicability of Hibiscus-van Gieson stain on the differential demonstration of collagen fibres. The new staining technique stains nucleus brownish-black and red blood cells, yellowish-brown. 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 collagen fibres. Details of the mechanism of staining need to be further investigated but the brown-black staining of the nucleus could be due to the anthocyanin-iron dye lake complex cum electrostatic interactions of the primary and counter stain. The local availability of hibiscus for use in the new technique and its affordability in a low resource setting makes it a potential choice alternative to the haematoxilin-dependent, HVG stain. The good results recorded from the exchange and substitution of one stain for the other (as demonstrated in B and C) testified to the flexibility of the Hibiscus-van Gieson technique. Interestingly too, its ability to resist fading over a long period of time is particularly encouraging. .

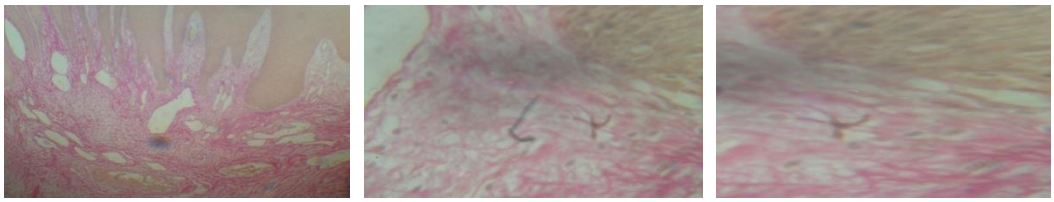
Figure 1: Comparison of Hibiscus van Gieson with Heamatoxylin van Gieson



H&E x40 H&E x100 H&E x100



HVG x40 HVG x100 HVG x100



Hib-VG x100 Hib-VG x400 Hib-VG x400

The histomorphological features of the skin, nucleus and collagen fibres are well shown in the Hib-VG when compared with the control HVG and H&E.

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