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THEOBROMA CACAO (COCOA) TREE STEM BACK EXTRACT AS A HISTOLOGICAL DYE FOR THE DEMONSTRATION OF GENERAL TISSUE STRUCTURE

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ABSTRACT

Introduction: Theobroma cacao is the taxonomic classification for the plant also called the cocoa tree and the cocoa tree, which is a small [(4 – 8m), (13 – 26ft)], evergreen tree in the family Malvaceae, native to the deep tropical regions of central and South America. Its seeds, cocoa beans, are used to make cocoa mass, cocoa powder and chocolate. **Aims and Objective:** To ascertain the suitability of Theobroma cacao (Cocoa) stem back tree extract as a histological stain and to compare the staining effects of Theobroma cacao (Cocoa) back tree extract with Eosin as a counterstain in histological staining procedures. **Materials and Method:** Tissue samples were gotten from the skin, kidney, stomach, lungs, liver and heart at post mortem examination. The tissues were histologically processed and sectioned with a rotary microtome. Theobroma Cacao (Cocoa) stem back tree was obtained from a show-boy cocoa farm in Ore, Ondo State, Nigeria. Theobroma Cacao (Cocoa) stem back was extracted in 2 (two) different ways; acqueous extraction and Methanolic Extraction, thereby giving rise to a crude extract, which was used as counterstain for haematoxylin. The control tissue sections were stained with haematoxylin and counterstained with eosin. **Result:** Theobroma Cacao (Cocoa) tree bark extract solution stained collagen fibres, muscle fibres and cytoplasm brown within 5minutes when it was used as a counterstain but methanolic extract was more intense in staining color than aqueous extract. **Conclussion:** Theobroma Cacao (Cocoa) stem back tree extract is a promising histological dye that is not only cheap but readily available which can serve as a useful alternative to eosin.

KEYWORDS: Theobroma-Cacao, Methanolic, Kidney, Liver, Stomach.

INTRODUCTION

A dye can simply be explained as a compound that impacts color on another substance. The advent of stain makes cellular differentiation of tissues more accurate because stains increase the optical differentiation of cellular element either by an alteration of contrast or impartation of color.[1] Based on the method of production, there are two types of dyes, synthetic and natural dyes.[1] Haematoxylin obtained from the Mexican tree called haematoxylon campechianum, is an example of a natural dye[2] that is widely used in histopathology, cytology, and in histochemistry, whereas eosin is a synthentic dye. Synthetic dyes are often efficient but may pose risk or hazards to human and animal health. This has resulted to the withdrawal and substitution of several dyes as their hazards became recognized.[3] The cocoa plant contains components that include: glutamic-acid, leucine, dopamine, fructose, glucose, phenylacetic-acid, linoleic-acid, lipase, lysine, niacin, peroxidase, alanine, alpha-theosterol, amylase, alkaloids, alpha-sitosterol, arginine, beta-carotene, calcium, ascorbic-acid, ascorbic-acid-oxidase, aspariginase, phenylalanine, phosphorus, theobromine, thiamin, riboflavin, rutin, tannins and many other important contents.[4] Cocoa beans contain fats, usually around 40%, consisting of cocoa butter, theobromine, starch, ash and protein matter (6% to 17%), small quantities of theine (0.05 to 0.37%).[5] The percentage varies because of the decomposition of glucosid influenced by fermentation. Theobromine may serve as a diuretic and has a stimulant or exciting action that is not possessed by chocolate itself. Theobromine and theophylline like caffeine, all are present in this cocoa plant, which are used in modern medicine as antiasthmatic.[5] With the worldwide concern over the use of eco-friendly and biodegradable materials, the use of natural dyes or stains obtained from plants have again

gained interest.[6] Moreover, as many developing countries can no longer afford the ever increasing cost of synthetic dyes, the use of cheaper, naturally occurring dyes from plants are being viewed as an alternative to synthetic dyes.

MATERIALS AND METHOD

Aqueous Extraction of Theobroma Cacao (Cocoa) Tree stem back staining solution: Back of theobroma cacao [cocoa] tree was gotten from show-boy cocoa farm in Ore, Ondo State, Nigeria. carefully cut about 500g of it into bits, put it in a plate, 500ml of distilled water was added, boiled for 5minutes which forced the staining component (dye), into the distilled water and turned deep brown, drained, filtered through filter paper on the funnel and the filtrate was used as counterstain, (secondary stain).

Methanolic Extraction of Theobroma Cacao (Cocoa) Tree stem back staining solution

The same method with aqueous extraction but differs in using 70% methanol instead of distilled water and hot air oven instead of stove because methanol is flammable.

Study Area

This research work was carried out at the department of Histopathology and Morbid Anatomy, Obafemi Awolowo University Teaching Hospital, ile-ife, Osun State and in Histopathology and Cytopathology Department, State Specialist Hospital, Akure, Ondo State.

Samples Collection and Preparations for Histology.

Human Tissue samples were gotten from the skin, kidney, stomach, lungs, liver and heart at post-mortem examination, the tissues were fixed in 10% formal saline for 24 hours, standard manual tissue-processing techniques were used. Sections of about 3µm were cut using a Slee Medical Rotary Microtome. The tissue sections obtained from the skin, kidney, stomack, lungs, liver and heart were grouped into three (3); the first group tissue sections were stained with haematoxylin (Harris Haematoxylin) and counterstained with Eosin; the second group oftissue sections were stained with haematoxylin (Harris Haematoxylin) as the primary stain and then counter stained with aqueous extract of Theobroma Cacao (Cocoa) Tree stem back; while the tissue sections in the third group were stained primarily with Haematoxylin (Harris Haematoxylin) and countrstained with methanolic extract of Theobroma Cacao (Cocoa) Tree stem back. The stained tissue sections of all the groups were viewed and analyzed with the aid of a light microscope for histological similarities and differences, thereafter, captured with Brunel light microscope, 20 mega pixels.

Staining Procedures (using Harris Haematoxylin and Theobroma Cacao (Cocoa) Tree stem back extract)

- Section were dewaxed in xylene for 15minutes
- Section were hydrated briefly through descending grades of alcohol e.g Absolute alcohol, 95%alcohol, 90%alcohol, 80%alcohol, 70%alcohol and distilled water
- Stained with Harris haematoxylin for 4minutes
- Section were then rinsed in running tap water
- Differentiated in 1% acid alcohol for 3second and latter blued in running tap water for 10minutes
- Sections were then counterstained with theobroma cacao tree stem back extract (methanolic extract solutions for 5 minutes and another sets of sections were counterstained with aqueous extract of Theobroma Cacao tree stem back solution for 5 minutes).
- Sections were finally rinsed in running tap water, dehydrated in ascending grades of alcohol, [70% alcohol, 80% alcohol, 90%alcohol, 95%alcohol, absolute alcohol].
- Cleared in xylene and mounted in a synthetic mountant.

Staining Procedure for control tissue sections (using Haematoxylin and Eosin staining technique)

- Section were dewaxed in xylene for 15minutes
- Section were hydrated briefly through descending grades of alcohol e.g Absolute alcohol, 95%alcohol, 90%alcohol, 80%alcohol, 70%alcohol and distilled water
- Stained with Harris haematoxylin for 4minutes
- Section were then rinsed in running tap water
- Differentiated in 1% acid alcohol for 3second and latter blued in running tap water for 10minutes
- Sections were counterstained with eosin for 2 minutes.
- Sections were finally rinsed in running tap water, dehydrated in ascending grades of alcohol, [70% alcohol, 80% alcohol, 90%alcohol, 95%alcohol, absolute alcohol].
- Cleared in xylene and mounted in a synthetic mountant.

RESULTS

PHOTOMICROGRAPHY

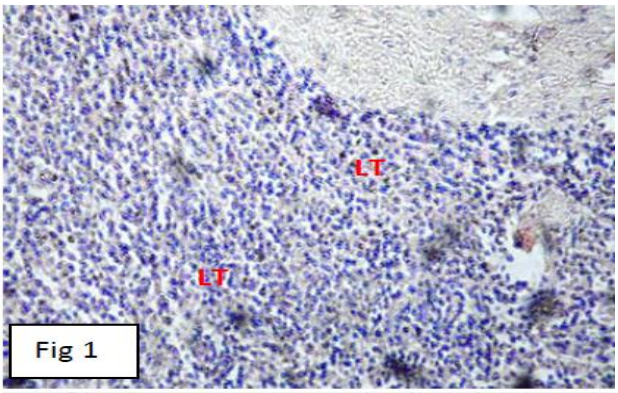

Fig 1 shows a Human tissue section of Nasopharynx stained with Haematoxylin and counterstained with methanolic extract of Theobroma Cacao Tree (Cocoa) stem back: LT= Lymphoid Tissue

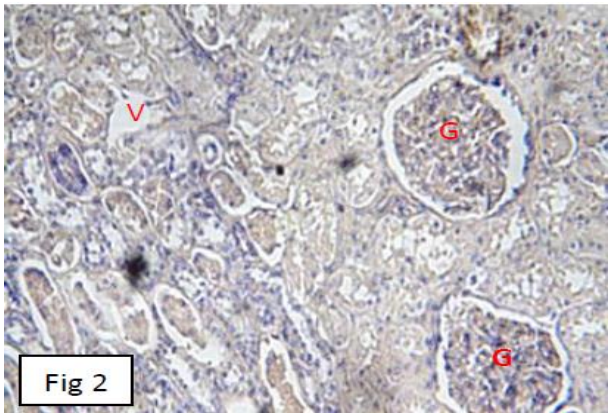

Fig 2 Shows a Human tissue section from human Kidney stained with Haematoxylin and counterstained with methanolic extract of Theobroma Cacao Tree (Cocoa) stem back: G= glomeruli, V= vein

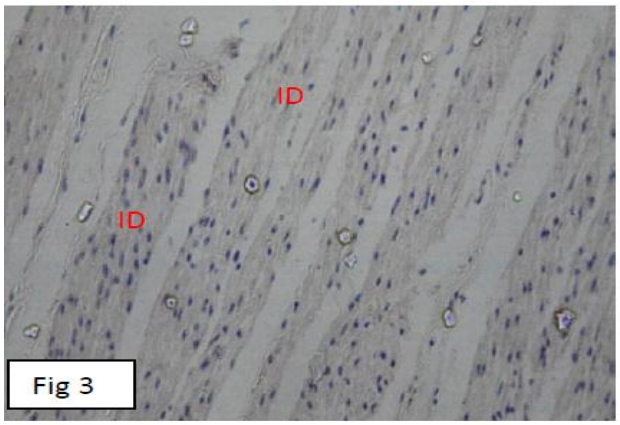

Fig 3 Shows a Human tissue section from human Heart stained with Haematoxylin and counterstained with Aqueous extract of Theobroma Cacao Tree (Cocoa) stem back: ID=Intercalated Discs

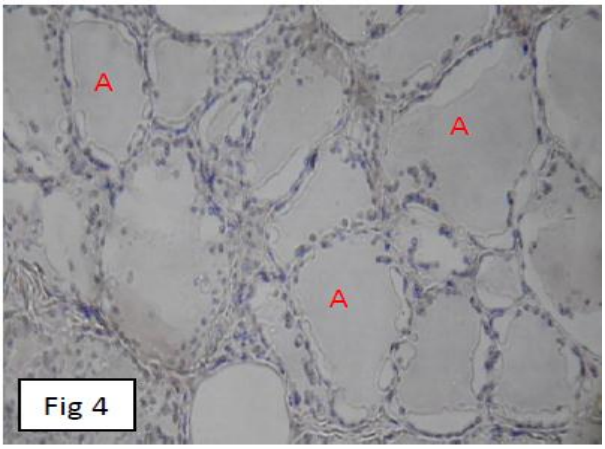

Fig 4 Shows aHuman tissue section from human Lungs stained with Haematoxylin and counterstained with Aqueous extract of Theobroma Cacao Tree (Cocoa) stem back: A=Alveoli

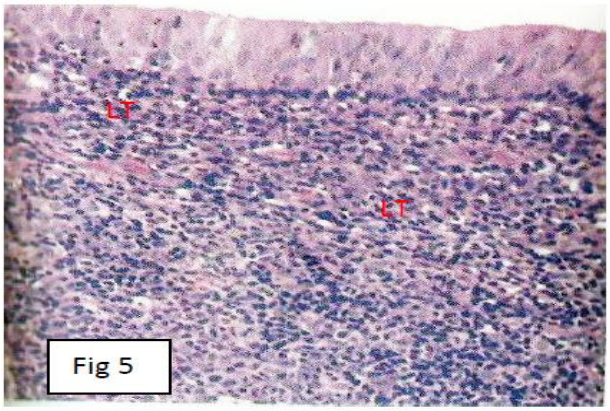

Fig 5 Shows a Control Human tissue section of Nasopharynx stained with Haematoxylin and counterstained with Eosin: LT= Lymphoid Tissue.

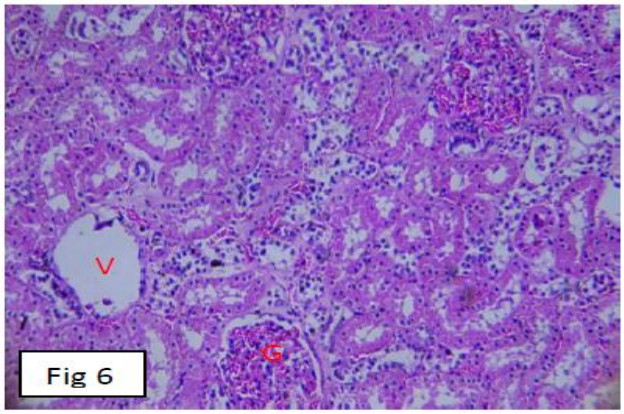

Fig 6 Shows a Control Human tissue section of Kidney stained with Haematoxylin and counterstained with Eosin: G=glomeruli, V=Vein.

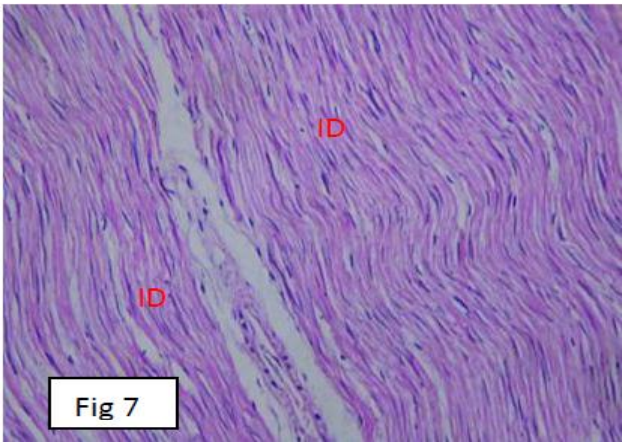

Fig 7 Shows a Control Human tissue section of Heart stained with Haematoxylin and counterstained with Eosin: ID= Intercalated Discs.

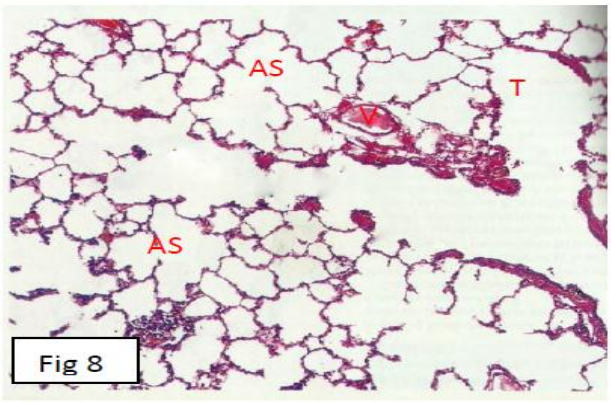

Fig 8 Shows a Control Human tissue section of Nasopharynx stained with Haematoxylin and counterstained with Eosin: AS= Alveoli Ring, T= Terminal Bronchioles

DISCUSSION

Theobroma Cacao tree (Cocoa) stem back extract contains several coloring compound. The ability of a dye to stain specific tissue structure is determined by certain factors, one of which is the acidity or alkalinity [basic] of the stain.[7] Acidic structure would be stained by basic dyes while basic structures would be stained by acidic dyes[1,2,8], owing to the strong affinity of theobroma cacao tree stem back extract dye for the cytoplasm, it can be deduced that the theobroma cacao tree stem back extract dye is acidic in nature because it stained muscle fibres and cytoplasm brown. Theobroma cacao contains flavonoids, which is typically polyphenolic compounds. Phenols are acidic due to their ability to release the hydrogen from their hydroxyl group[9], hence this now gives theobroma cacao tree stem back extract solution the ability to stain the basic parts of the cell. When extract of theobroma cacao tree (Cocoa) stem back was used as a counter stain for haematoxylin, the nuclei took the blue coloration which enables a clear contrast to be made between the different structures of the cells, the muscle fibres and cytoplasm stained brown within 5minutes. It was also observed that tissue section stained with methanolic extract of theobroma Cacao tree (Cocoa) stem back appeared to be more intense and clearer compared to tissue section stained with Aqueous extract of Theobroma Cacao tree (Cocoa) Stem back. The reaction of theobroma cacao tree (cocoa) stem back extract stain is similar to the reaction of eosin in the haematoxylin and eosin techniques except for its intense brown coloration. For this reason, the theobroma cacao tree (cocoa) stem back extract was tried as a counter stain because of its high pigment content and used as a substitute for eosin in the haemtoxylin and eosin technique.[1]

CONCLUSION

In conclusion, theobroma cacao tree bark 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.

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