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HISTOMORPOHOLOGICAL ASSESSMENT OF THE EXTRACT OF THE STALK OF SORGHUM BICOLOR AS A COUNTERSTAIN

[1]Omoowo BT, [1]Bankole JK, [2]Muhammed AO, [3]Benard SA, [4]Afolabi OO

1. Department of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria
2. Department of Histopathology, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Sokoto State Nigeria
3. Histopathology Unit, Department of Pathology, University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria
4. Department of Histopathology, College of Health Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria

Corresponding Author: Muhammed AO
Email: muhammedrashola@gmail.com

ABSTRACT

Aim: The objective of this study was to investigate the staining ability of the extract of the stalk of Sorghum bicolor as a counterstain on Haematoxylin.

Methods: Tissue blocks of trachea, oesophagus and ileum were retrieved from the Autopsy Block
Archive of the Department of Pathology, University of Ilorin Teaching Hospital Ilorin.
Eight (8) serial sections labeled A to H were made from each block and stained with Harris haematoxylin. Section A was counterstained with 1% ethanolic eosin, as control. Different preparations of extracts of the stalk of Sorghum bicolor were used to counterstain sections of Groups B to H as follows: B; 5% aqueous extract with 0.5g Potash, C; 5% aqueous extract, D; 5% extract in 70% ethanol, E; 10% extract in 70% ethanol, F; 5% extract in absolute ethanol, G; 10% extract in absolute ethanol and H; 5% aqueous extract with 40µl of acetic acid.

Results: The stalk extracts of S. bicolor stained the cytoplasm in shades of pinkish brown

Conclusion: This study established the cytoplasmic counter-staining ability of stalk extracts of S. bicolor, and the 10% extract in absolute ethanol had the closest resemblance to the Group A, the control group. It is therefore suggested that 10% extract in absolute ethanol can be substituted for eosin due to its domestic availability, ease of preparation and use, and above all, its good cytoplasmic contrast with the nuclear stain.

Keywords: Sorghum bicolor, Counterstain, Extract, Eosin

INTRODUCTION

Sorghum bicolor is a member of grass family (Poaceae) and can thrive in hot areas with little rainfall, providing nutrients for millions of people (Bukantis, 1980). It is one of the world's leading cereal crops (Bukantis, 1980). 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 (Umar, 2009). Sorghum bicolor is typically an annual, but some cultivars are perennial. It grows in clumps that may reach over 4 meters (Umar, 2009). The grain is small, ranging from 3 to 4mm in diameter; the leaves and stem are covered with wax layer (Umar, 2009). Sorghum is an important staple food and also an important feed grain and fodder crop. It is used in beer making; it is also distilled to make a popular spirit and vinegar (Watt and Breyer-Brandwijk, 1962). 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) (Balole and Legwaila, 2005). In some West African countries, the dye can be extracted from the plant to color leathers, cloths, calabashes and as body pigment (Cobley and Steele, 1976). Sorghum is used largely for forage in the U.S. It is very important in the world as part of human diet, with over 300 million people dependent on it (Bukantis, 1980). The stems of sweet sorghum are chewed like sugarcane and for sweet syrup. Sorghum plant residues are used extensively as materials for roofing, fencing, weaving and as fuel (Balole and Legwaila, 2005). The use of Sorghum bicolor leaf sheath as a remedy for anaemia by traditional medicine healers is common in Nigeria particularly within the local people of the Yoruba and Hausa tribes (Akande et al., 2010). It is used in arrow poisons. The red pigment is said to have antimicrobial and antifungal properties. Malted Sorghum bicolor grain is higher in protein and lower in fat content than corn and this is partly responsible for its medicinal potential (Makokha et al., 2002). Avwioro et al., (2009), in their study on the Biochemical observations on Wistar albino rats fed with dye extracted from Sorghum bicolor leaf reported that the serum sodium, potassium, and urea increased progressively with increase in concentration of the extract, while the bilirubin levels did not change remaining at <17mmol/l. Stains are substances, which colour tissues in order to aid optical differentiation of tissue components. The mechanisms of staining of some tissues are histochemical in nature (Avwioro, 2010). Tissues are made up of organic and inorganic compounds which are capable of undergoing chemical reactions in vitro to terminate in opaque or coloured end products, (Avwioro, 2010). Therefore, sections of human tissue appear dull and uninteresting because they lack contrast due to similar refractive index and colour when viewed under the microscope (Cellpath, 2006). The binding of dyes to tissues is no different to any other chemical bonding and the mechanisms rely on the same binding forces that occur in all other organic compounds. The dye must form some type of bond or link to the tissue or they will simply be rinsed out of the tissue when the section is washed in another reagent. (Cellpath, 2006).The commonest stain in use in histopathology laboratories all over the globe is the haematoxylin and eosin (H&E) which colour the nuclei a dark blue or purple and stains the cytoplasm and connective tissue in shades of pink (Cellpath, 2006). Stains can be used in a variety of ways and for specific purposes, which enable a diagnosis to be arrived at (Avwioro, 2006). The extraction and application of colouring matter from the stalk of Sorghum bicolor which usually wastes away after harvest will, therefore, be of great contribution to the exploitation of natural dyes and their applications. This research is to contribute to the use of natural dye from Sorghum bicolor as a substitute for eosin in the staining of human and animal tissue in diagnostic histopathology.

MATERIALS AND METHODS

Sample Collection

Tissue blocks of trachea, oesophagus and ileum retrieved from the Autopsy Block Archive of the Department of Pathology, University of Ilorin Teaching Hospital were used for this study. Ethical approval was obtained from The University of Ilorin Teaching Hospital Medical Laboratory Services Ethical Committee (MLSETCO).

Processing and Extraction of Sorghum Bicolor Stalk Staining Solutions

The sorghum bicolor stalk was bought from a local market (Oja titun) in Ilorin, Kwara State and taken to the Department of Plant Science of University of Ilorin for identification and authentication. It was milled into powdery form

with an electric blender Maxwell Model W55110V. 5g of the powder was measured into seven different containers, and 0.5g of potash alum was measured separately as well, using a sensitive weighing balance (Ohaus).

Extraction with Distilled Water

5 grams of the powder was dissolved in 100ml of distilled water and heated on a Bunsen burner to boil for 10 minutes. The solution was filtered with filter paper and the filtrate was poured in a plastic container giving 5% solution.

Extraction with Distilled Water and Potash Alum

5 grams of the powder was dissolved in 100ml of distilled water and 0.5g of potash alum was added and heated to boil for 10 minutes. It was filtered and poured in a plastic container.

Extraction with 70% and Absolute Ethanol

5 grams of the powder was dissolved each in four different plastic containers of 50ml and 100ml of 70% and absolute ethanol respectively. They were allowed to stay for 24 hours at room temperature (25±2ºC), then filtered and the filtrates were poured into plastic containers to give 5% and 10% concentration of 70% and absolute ethanol staining solution.

Aqueous Extraction and Acetic Acid

5 grams of the powder was dissolved in 100ml of distilled water, heated to boil for 10 minutes. It was filtered and poured in a plastic container. 40µL of acetic acid was added to the final staining solution

Slide Preparation and Staining

Eight (8) serial sections labeled A to H were made from each block, stained with Harris haematoxylin and counterstained with different preparations and concentrations of S. bicolor stalk extract as follows:

A. 1% ethanolic eosin stain (Control)
B. 5% aqueous extract with 0.5g potash
C. 5% aqueous extract
D. 5% extract in 70% ethanol
E. 10% extract in 70% ethanol
F. 5% extract in absolute ethanol
G. 10% extract in absolute ethanol
H. 5% aqueous extract with 40µl of acetic acid

Staining Procedure for Test

Harris haematoxylin and the eight groups of Sorghum bicolor extracts were used to stain tissue sections.

Method

1. Sections were dewaxed in xylene, dehydrated in alcohol and taken to water
2. Stained in Harris haematoxylin for 10 minutes
3. Rinsed in water
4. Differentiated in 1% HCl in 70% alcohol
5. Blued in running tap water for 10 minutes
6. Counterstained with different concentrations of S. bicolor for 3 minutes
7. Dehydrated with alcohol, cleared in xylene and mounted with DPX

Staining Procedure for Control (Haematoxylin and eosin)

Method

1. Sections were dewaxed in xylene, dehydrated in alcohol and taken to water
2. Stained in Harris haematoxylin for 10 minutes
3. Rinsed in water
4. Differentiated in 1% HCl in 70% alcohol
5. Blued in running tap water for 10 minutes
6. Counterstained with 1% alcoholic eosin for 3 minutes
7. Dehydrated with alcohol, cleared with xylene and mounted with DPX

RESULTS

Photomicrographs of the sections stained with the extracts of S. bicolor

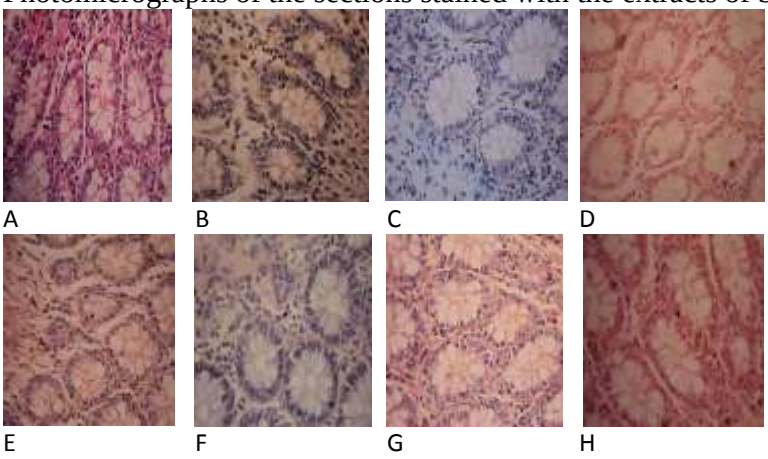

Fig. 1. Ileum X100

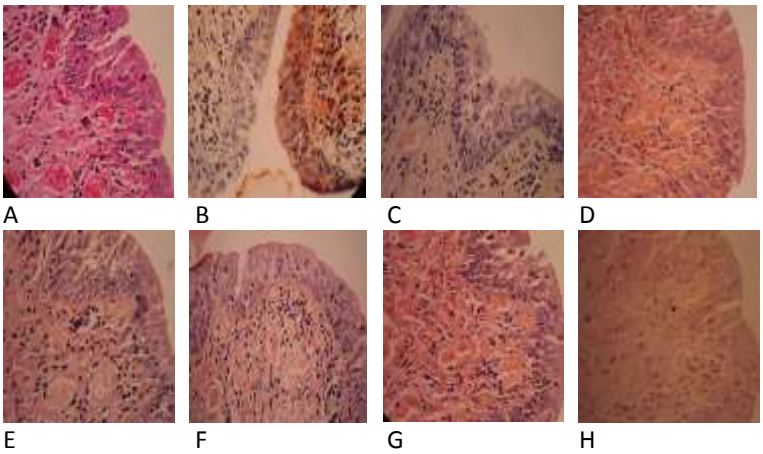

Fig. 2. Trachea X100

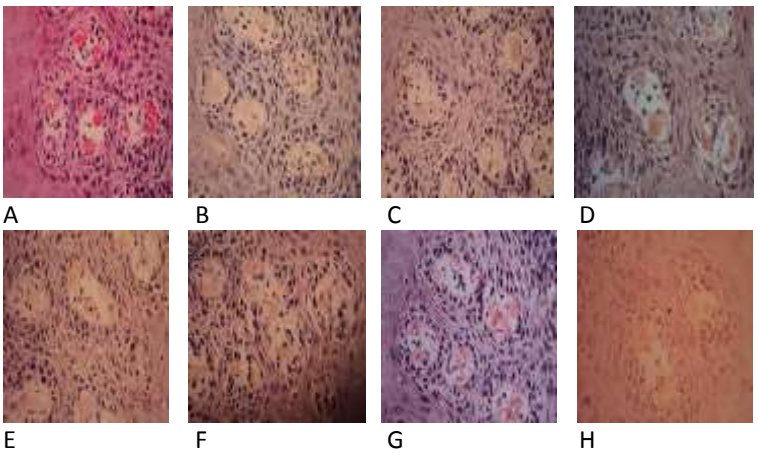

Fig. 3. Oesophagus X100

Cytoplasm of Groups B to H stained in shades of pinkish brown. The nuclear staining was decolourised in Group H of the entire organs after counterstaining and Group G has the closest morphological resemblance to the control group (Group A) which stained a pink colour across the entire groups.

DISCUSSION

Earlier researchers did not concentrate on the staining ability of S. bicolor as a histopathological counter-stain. Sorghum bicolor has been used extensively as food (Watt and Brayer-Brandwijk, 1962), as a remedy for anaemia (Ogwumike, 2002) and medicines for several ailments (Watt and Brayer-Brandwijk, 1962; Morton, 1981). It has also been used as a dye for clothes, leather and calabashes (Cobley and Steele, 1976). Avwioro et al., (2006), did appreciable work on the leaf extract of the plant and reported that ethanolic extracts of the leaf stained muscles, collagen fibres and red blood cells in shades of pinkish-yellow. Routine eosin stain of the control was used to compare the cytoplasmic staining of other groups (B to H). Eosin dye stained the cytoplasm pink in all the organs, and the blue colour of the nuclei was well preserved. Extract of Groups B to H stained the cytoplasm of all the organs pinkish brown and also preserved the nuclear staining of the tissues. Group H extract however, bleached the dark blue colour of the primary stain (haematoxylin) from all the nuclei. Both nuclei and cytoplasm therefore appeared blemishly pink. This may be due to the addition of acetic acid to the extracts of that group. Extract of Group G (10% S. bicolor) has distinct pink colouration that is comparable to the control. In this study, extracts of Sorghum bicolor stalk dye stained the cytoplasm excellently well, and preserved the tissue constituents distinctly, though the colour impacted on cytoplasm varies slightly from the control, group A. These variations can be attributed to differences in the extraction solvents, concentrations, temperatures and pH. While the staining characteristics of the stalk extracts of S. bicolor have been established in this study, it is worth noting that 10% S. bicolor stalk extract of absolute ethanol has the closest morphologic resemblance and may be substituted for eosin in diagnostic histopathology staining.

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REFERENCES

Akande, IS, Oseni, AA, Biobaku OA (2010). Effects of aqueous extract of Sorghum bicolor on hepatic, histological and hematological indices in rats. Journal of cell and Animal Biology. 4(9):137-142.

Avwioro, OG (2010). Staining. In: Histochemistry and tissue pathology. 2nd edition, Nigeria: Claverianun Press, 132-155.

Avwioro, OG, Aloamaka, CP, Oduola, T and Komolafe, AO (2006). The Effect of Some fixatives on the staining ability of Sorghum bicolor extracts on tissue sections. African Journal of Biotechnology. 5(22): 2326-2328.

Avwioro, OG, Aloamaka, CP, Oduola, T and Komolafe, AO (2006). Collagen and muscle stains obtained from Sorghum bicolor. Sc. J. Clin. Lab. Invest. 66: 161-168.

Avwioro, OG, Owoloagba, GK, Anibor, E, Bankole, JK, Oduola, T, Adeosun GO and Aloamaka, CP (2009). Biochemical observations in wistar rats fed with the histological dye extract from S. bicolor. Int J. Med. Sci, 1 (10):464-466.

Balole, TV and Legwaila, GM (2005). Sorghum bicolor (L.) Moench (Internet) record from protabase. Jansen. P.C.M and Cardon D. (Editors).

Bukantis, R (1980). Energy inputs in sorghum production. In: Handbook of energy utilization in agriculture. p. 103–108. In: Pimentel, D. (ed.). CRC Press, Inc. Boca Raton, FL

Cellpath (2006). In: Staining theory. pp 67-72

Cobley LS, Steele WM (1976). An introduction to the botany of tropical crops. 2nd ed. pp 43-45

Makokha, AO, Oniango RK, Njoroge SM, Kinyanjui PK (2002). Effect of malting on protein digestibility of some Sorghum (Sorghum bicolor) varieties grown in Kenya. African. J. Foods Nutr. Sci., 2(2): 168-175.

Morton, JF (1981). Atlas of medicinal plants of Middle American. Bahamas to Yucatan. CC Thomas, Springfield, IL

Ogwumike, OO (2002). Hemopoietic effect of aqueous extract of the leaf sheath of Sorghum bicolor in Albino rats. Afri. J. Biomed. Res. 5:69–71

Umar, A (2009). Effect of Raw and Modified Sorghum Cellulose Fibre on the Mechanical Properties of Natural Rubber Composite. MSc. Dissertation. Dept. of Pure and Industrial Chemistry (B.U.K).

Watt, JM and Breyer-Brandwijk, MG (1962). The medicinal and poisonous plants of southern and eastern Africa. 2nd ed. E & S. Livingstone, Ltd., Edinburgh and London