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SORGHUM BICOLOR EXTRACT: A SUITABLE COUNTER STAIN IN HIBISCUS EXTRACT NUCLEAR STAINING OF LIVER AND KIDNEY

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ABSTRACT

Aim: To investigate the suitability of alcoholic extract of Sorghum bicolor stalk as a counter stain for Hibiscus extract stained nuclei.

Method: Formalin fixed kidney and liver biopsies were processed by the paraffin wax technique, sectioned, stained with Hibiscus extract solution and counterstained with 10% alcoholic extract of Sorghum bicolor stalk. Parallel sections were stained with H&E as control.

Results: Results show distinct dark - violet nuclear staining with light-brown staining of collagen and other components.

Conclusion: Sorghum bicolor stalk alcoholic extract could be a useful substitute to eosin in histology for the demonstration of collagen and other cytoplasmic components especially when H. sabdariffa is used as the nuclear stain.

Keywords: Hibiscus, Nuclear staining, Sorghum bicolor extract

INTRODUTION

Sorghum bicolor is a member of the grass family (poaceae) and can thrive in hot areas with little rainfall, providing nutrients for millions of people. 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 in height (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 BreyerBrandwijk, 1962). Sorghum bicolor has a red dye present in the leaf sheath and sometimes also in adjacent stem parts. In Africa, this dye is used for goat-skin leather (Nigeria), in local weaving design (Sudan) (Balole and Legwaila, 2005). The dye is used in dyeing. 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. 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 high in protein and low in fat content than corn and this is partly responsible for its medicinal potential (haemopoietic ability) (Makokha et al., 2002). Sorghum stalk extract has been used as a counter stain in the staining of cytoplasmic components of tissues with promising outcomes (Omoowo et al., 2014). Hibiscus sadariffa belong to the family Malvaceae, which is commonly called roselle. Roselle is cultivated in India, Malaysia, Tropics, subtropics and Central America (Roselle, 1987, Durance et al., 1999). The plant is widely grown in Nigeria and other sub-Saharan African countries as a crop used for demarcation of farm plots. The aqueous extract of the dry red calyx is often prepared as a drink for refreshment locally called 'zobo'. Other uses of the calyces include its being an edible vegetable among the Yoruba ethnic group of South-West, Nigeria and a natural food colorant. (Aballa et al., 1993: Adegunloye et al., 1996). Egyptians use to drink hibiscus extract and call it karkadae, while in Iraq, it is called red tea. Studies in Iraq suggest using of hibiscus extract in food industry, using it as a syrup and coloring agent (Alzubaidi, 1977). Hibiscus was found as a natural source of pectin, which solidifies jelly and ice cream preparations (Ali, 2000). Hibiscus calyces contain per 100gm of edible portion, calcium (1.263mg), niacin (3.765mg), riboflavin (0.277mg) and iron (8.98mg) (Durance et al, 1999). Chemical analysis in Iraq (Muller et al., 1992) of Kujarat reported their values of 100mg of Ca and 9.55mg of Fe per mg of dry matter. Three water-soluble polysaccharides have been isolated from flower buds of Hibiscus sabdariffa (HIB, 1.2.3) (Muller et al., 1992). Medical uses of these flowers are wide. Infusion of calyces is regarded as diuretic, choleretic, febrifugal, hypotensive, decreasing the viscosity of blood and stimulating intestinal peristalsis (Roselle, 1987). Other medical studies proposed its effect in protection from induced cytotoxicity and genoetoxicity by different mechanisms (Tesnget al., 1996). Other researchers proposed its use as a natural stain. They used the extract to stain blood film, fungi and plant tissue (Al –Sarraj et al., 1997). Dried calyces of Hibiscus have dark red pigment. They contain flavonoids gossypetine, hibiscetine and sabdaretine. The major pigment reported as hibiscin that was identified as daphniphylline. Small amount of delphinidine 3-monoglucosides, cyanidin 3-monoglucosides (chrysanthenin) and delphinidine are also present (Roselle, 1987: Cardon, 2007). Mordanted Hibiscus sabdariffa solution has been used to demonstrate nuclear component of tissues according to the works of Benard, (2008) and Egbujo et al., (2008). However, to the knowledge of the authors, no work has been done on the suitability of Sorghum bicolor extract as a counter stain in Hibiscus extract nuclear stain. The histo-morphological integrity of nucleus and cytoplasm if preserved by the application of the two local dyes will add to knowledge on iso-electrically compatible local dyes that are suitable for use in histological demonstration of tissue components.

MATERIALS AND METHODS

Dry leaves of Hibiscus sabdariffa were purchased in a local market in Ilorin, Kwara State, Nigeria and processed as recommended by Benard, (2008). In the same vein, Sorghum bicolor stalk was locally obtained and processed in line with an earlier recommendation by Omoowo et al., (2014). 10% Fomalin fixed, paraffin wax processed liver and kidney tissues were sectioned at 4 microns and stained with Hibiscus extract solution, counter stained with 10% alcoholic Sorghum bicolor solution. Parallel sections were stained with H&E as controls.

Preparation of 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 colored 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

Preparation of 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.

Hibiscus Extract/Sorghum Staining Procedure

1. Dewax in xylene and hydrate through 100%, 90%, 70%, 50% alcohol to water
2. Stain in Hibiscus extract solution for 5 minutes
3. Wash in running tap water for 2 minutes
4. Counter-stain in 10% alcoholic Sorghum for 3 minutes
5. Dehydrate in ascending grades of alcohol
6. Clear in xylene
7. Mount in DPX

H&E Staining Procedure

1. Dewax in xylene and hydrate through 100%, 90%, 70%, 50% alcohol to water
2. Stain section in Harris haematoxylin for 15 minutes
3. Rinse in water
4. Differentiate in 1% acid alcohol
5. Blue in running tap water for 10 minutes
6. Counter-stain in 1% alcoholic eosin for 60 seconds
7. Dehydrate in ascending grades of alcohol
8. Clear in xylene
9. Mount in DPX

RESULTS

Results show iso-electrical compatibility of the two stains. Nuclear component appear dark-violet while cytoplasmic components appear light-brown in the Hibiscus-Sorghum combination. The histomorphological features of the bowman's capsules and glomerulus of the kidney section were well preserved with clear demarcation of cellular boundaries comparable with the H&E stained sections (Fig Ia & IIa). The liver sections also show similar staining appearance. Liver cell nuclei were stained dark-violet while the cytoplasm appears light-brown (Fig. Ib). The histomorphological features of the liver were also well preserved similar to H&E section (Fig. Ib&IIb).

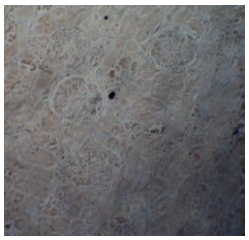

Fig Ia: Hibiscus/Sorghum. Kidney showing dark-violet nucleus and prominent Bowman's capsule x400

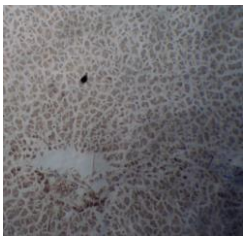

Fig Ib: Hibiscus/Sorghum. Liver showing dark-violet nucleus and light-brown cytoplasm x 400

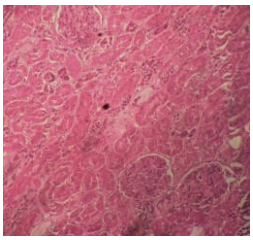

Fig IIa: Kidney showing purple-blue nucleus and prominent Bowman's capsule membrane. H&E x 400

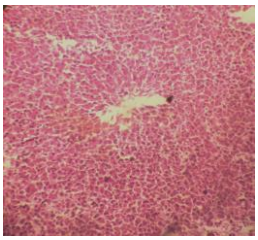

Fig II b: Liver showing purple-blue nucleus and pink cytoplasm. H&E x 400

DISCUSSION

Few works have been done so far on the staining ability of S. bicolor stalk extract. Earlier on, alcoholic extract of the leaf was applied in the staining of muscles, collagen fibres and red blood cells resulting in shades of pinkish-yellow (Avwioro et al., 2006). In a recent novel report, 10% alcoholic extract of the stalk of S. bicolor was applied as counter stain in combination with haematoxylin nuclear stain to give results similar to standard H&E and thereafter suggested as a substitute for eosin (Omoowo et al., 2014). In this work, Sorghum bicolor stalk extract is confirmed as a substitute for eosin. The staining results obtained presents different shades of colour distinct from the standard haematoxylin and eosin. The histological characteristics of the tissues were however well preserved to details similar to H&E stained sections. The nuclei appear distinct and well stained with a dark-violet colour. The cytoplasm and other components appear light - brown. In essence, both Hibiscus extract solution and 10% alcoholic extract of the stalk of S. bicolor are iso-electrically compatible. This new staining technique could therefore be a substitute to H&E staining technique in general tissue demonstration. The staining combination shows promise as available local dyes in the demonstration of nuclear and cytoplasmic contents of tissues and could be useful in the histopathological diagnosis of diseases.

CONCLUSION

Alcoholic extract of S. bicolor stalk is suitable as counter stain in Hibiscus extract nuclear staining.

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