African Journal of Biotechnology Vol. 5 (22), pp. 2326-2328, 16 November, 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

Short Communication

The effect of some fixatives on the staining ability of *Sorghum bicolor* extracts on tissue sections

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Accepted 20 October, 2006

The effect of some fixatives on the staining reactions of the extracts of *Sorghum bicolor* on tissue sections was studied in order to identify the most appropriate fixative for the stain. Tissue sections taken at postmortem were fixed in 10% formol saline, Carnoy's fluid, Bouin's fluid, Formol sublimate, Heidenhain's susa and Zenker's fixative. The sections were subsequently stained with Weigert's haematoxylin and counterstained in acidified and neutral alcoholic extracts of *S. bicolor*. The staining reactions after the use of all fixatives were the same; collagen fibres, muscles and red blood cells were stained in shades of pinkish-yellow. The active staining constituent of *S. bicolor* had previously been described as apigeninidin, a type of flavonoid.

Key words: *Sorghum bicolor*, fixative, stain, flavonoid.

INTRODUCTION

The staining ability of ethanolic extracts of the leaves of *Sorghum bicolor* L. Moench on tissue sections had previously been described (Avwioro et al., 2006). The alkaline mixtures did not stain any of the tissues used but the acidic and neutral alcoholic mixtures stained collagen fibres, muscles and red blood cells in shades of pinkish-yellow. Good contrast was obtained when nuclei were pre-stained with Weigert's haematoxylin (Avwioro et al., 2006). The active staining compound in *S. bicolor* had been extracted and found to be a flavonoid (Avwioro et al., 2006), which was previously described as apigeninidin, (3-deoxypelargonidin) by Kouda-Bonafos et al. (1998).

S. bicolor L. Moench is an annual, erect, 0.6–5 m tall summer plant. The leaves are broad and corn-like but with shorter and wider blades that are glabrous and waxy with overlapping margins (Bukantis, 1980). Sorghum is important in the human diet world-wide, with over 300 million people dependent on it. It is also used largely for forage in the US. The leaves are also of some local medicinal importance. For example, it is widely used among West Africans to make local antibiotic syrup (Bukantis, 1980). It is also used as a colouring flavour in cooking some species of white cowpea.

Sometimes the choice of a fixative for tissues influences subsequent histochemical reactions (Culling, 1974). This is because fixatives are chemicals and they react with tissue structures to produce new compounds, which are not only capable of withstanding autolysis and putrefaction but are capable of preparing the tissue for histochemical reactions. Generally, fixatives which contain acetic acid, favour the staining of nucleus (Avwioro, 2002). Formalin, a major constituent of 10% formol saline supports many staining techniques. Carnoy's fluid, which contains alcohol and acetic acid, is a good fixative for chromosome studies. Zenker's fluid is good for the trichrome methods for collagen fibres, while Helly's fluid is said to be a good fixative for micro-anatomical and cytological studies (Avwioro, 2002). Several fixatives are currently used in histopathology, each of them with its own merits and demerits. Some of them are specific for

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certain histological techniques, although they may be used for other techniques. The aim of the study was to identify the most appropriate fixative for use with the extract of *S. bicolor* as a stain.

MATERIALS AND METHODS

Preparation of fixatives

Six fixatives were prepared. These were (i) 10% formol saline, prepared as follows: 10 ml formalin, 0.9 g sodium chloride and 90 ml tap water. (ii) Carnoy's fluid prepared as follows: 60 ml absolute alcohol, 30 ml chloroform and 10 ml glacial acetic acid. (iii) Bouin's fluid was prepared as follows: 75 ml saturated aqueous picric acid, 20 ml formalin and 5 ml glacial acetic acid. (iv) Formol sublimate prepared as follows: 90 ml saturated aqueous mercuric chloride and 10 ml formalin. (v) Heidenhain's susa prepared as follows: 4.5 g mercuric chloride, 0.5 g sodium chloride, 2 g trichloroacetic acid 4 ml acetic acid, 20 ml formalin and 75 ml distilled water. (vi) Zenker's fixative prepared as follows: 5 g mercuric chloride, 2.5 g potassium dichromate, 1 g sodium sulphate, 95 ml distilled water and glacial acetic acid added just before use.

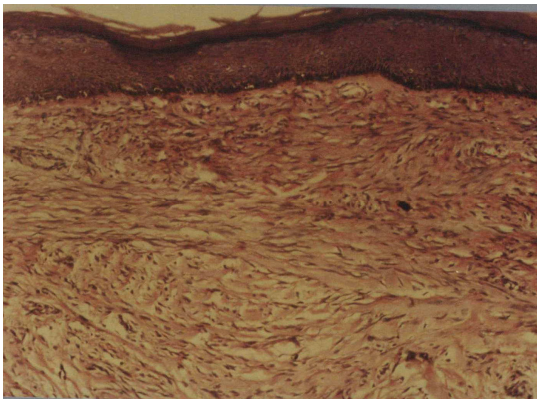

Figure 1a. A section of skin stained with extracts of *S. bicolor* prefixed with the fixatives (X250). All tissue sections appeared the same and the differences between them could not be discerned.

Preparation of sections

Thin slices of human tissues, 3 mm thick, were obtained from the skin, liver, intestine, kidney and lung at post-mortem. They were fixed for 24 h in 10% formal saline, Carnoy's fluid, Bouin's, Formol sublimate, Heidenhain's and Zenker's fixative and thereafter processed for embedding in paraffin wax using the automatic tissue processor (obtained from Sakura Fine Tech, The Netherlands) by dehydrating through 70, 90, 95% and two changes of absolute ethanol for 1 h 30 min each. Clearing was done through changes of xylene twice, for 2 h each, infiltrating through two changes of paraffin wax at 70°C for 1 h 30 min each and embedded in paraffin wax. Sections were cut at 4 µm with the rotary microtome (Sakura Fine Tech), attached to slides and dried at 65°C for 45 min.

Preparation of *S. bicolor* leaf extract

Fresh leaves of *S. bicolor* were collected from the botanical garden of Obafemi Awolowo University Ile Ife, Nigeria, rinsed several times in distilled water and drained. The leaves were cut into tiny pieces and dried in an open-air oven (Gallenkamp, England) at 60°C for 72 h. The leaves were milled until they became fine powder; 2.5 kg of the powdered plant material was extracted with 2 L of 70% ethanol under soxhlet for 72 h until completion. The extract was filtered and concentrated in vacuo at 50°C and finally dried in a desiccator to obtain a secondary fine powder.

Preparation of *S. bicolor* staining solutions

Five grams of the concentrate was dissolved in 100 ml each of 70% alcohol and 1% glacial acetic acid in 70% alcohol. The mixtures were boiled in a water bath for 5 min to dissolve the solutes completely.

Staining with solutions of *S. bicolor*

The sections were dewaxed in xylene, hydrated through graded solutions of alcohol and stained in Weigert's iron haematoxylin for 10 min. They were then differentiated in 1% acid alcohol for 2 – 5 s and rinsed in tap water. The sections were then counterstained separately with solution of *S. bicolor* extract in 70% alcohol and in solution of *S. bicolor* extract in 1% glacial acetic acid in 70% alcohol for 10 min. The sections were finally differentiated in 1% acid alcohol for 10 s, rinsed in water for 10 min, dehydrated through ascending grades of alcohol, cleared, and then mounted in a synthetic mountant.

Control sections

Control tissue sections were taken from the tissues fixed in 10% formol saline for 24 h. They were processed as earlier described and the sections stained for general tissue structure with haematoxylin and eosin, and with *S. bicolor* extracts as described above.

RESULTS AND DISCUSSION

There was no significant difference in the staining reactions of the extracts of *S. bicolor* on tissue sections pre-fixed in 10% formol saline, Carnoy's fluid, Zenker's fluid, Helly's fluid and Bouin's fluid. There was also no significant difference in the staining time and the intensity of the stain on tissues pre-fixed in the different fixatives. The speed of differentiation with acid alcohol and dye fastness on tissue sections were the same after all the fixatives used. The acidic and neutral alcoholic mixtures of *S. bicolor* stained collagen fibres, muscles and red blood cells in shades of pinkish-yellow after all the fixatives used. Nuclei appeared black on all the sections on account of the Weigert's haematoxylin nuclear stain (Figures 1a,b,c).

The objective of fixation is to preserve cells and tissue constituents in as close a life-like state as possible. Fixation stops autolysis and bacterial decomposition and stabilises the cellular and tissue constituents so that they can withstand tissue processing and subsequent staining. Some fixatives act as mordants (Avwioro, 2002). A good example of this is potassium dichromate, which is used

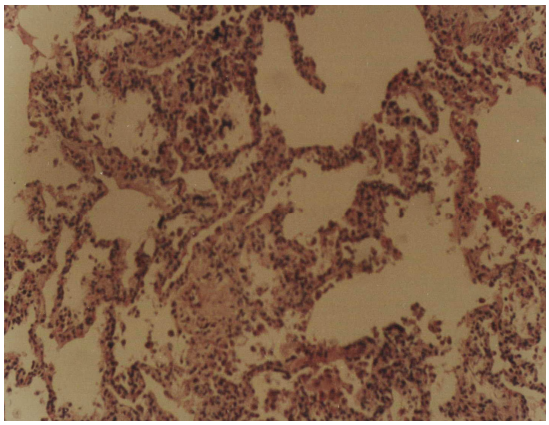

Figure 1b. A section of lung stained with extracts of *S. bicolor* prefixed with the fixatives (X250). All tissue sections appeared the same and the differences between them could not be discerned.

Figure 1c. A section of lung stained with extracts of *S. bicolor* prefixed with the fixatives (X250). All tissue sections appeared the same and the differences between them could not be discerned.

for chromatisation of tissue sections when mitochondria are to be stained by Mallory's phosphotungstic acid haematoxylin and by Heidenhain's iron haematoxylin (Carleton et al., 1976). The effect of such fixatives, which contain metallic salts, which could act as mordants were not significant when extracts of *S. bicolor* were subsequently used to stain the tissue sections. Most staining techniques do not require pre-treatment with special fixatives. It can be said that the stain extracted from *S. bicolor* belongs to this group and does not require pre-treatment of tissue sections with special fixatives.

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