Curcuma Longa: Staining Effect on Histomorphology of the Testis

Rosemary B Bassey¹, Ademola A Oremosi², Abraham AA Osinubi²

¹Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Akwa Ibom State, Nigeria; ²Department of Anatomy, College of medicine, University of Lagos, Lagos, Nigeria

Abstract

Background: The use of non-allergic, non-toxic and eco-friendly natural dyes has become a matter of significant importance due to the increased environmental awareness in order to avoid some hazardous synthetic dyes.

Method: The ethanolic extract of Curcuma longa was diluted using 1% acetic acid in 70% ethanol to a concentration of 0.2 g/ml. It was used to stain histological sections of the testes for 15 minutes. Curcuma longa was also used as a counter stain for Haematoxylin. Phytochemical constituents were investigated.

Results: The Curcuma longa dye distinctly stained the seminiferous epithelium and interstitium yellow. Curcuma longa provided a good counter stain for Haematoxylin, taking up the acidic staining characteristics with Haematoxylin staining the basic staining characteristics. Phytochemical screening revealed the presence of saponins, alkaloids, tannins and flavonoids.

Conclusion: Curcuma longa has good potential for use as a counter stain for Haematoxylin in the staining of tissues in lieu of Eosin.

Introduction

Colour is one of the elements of nature that has made human living more aesthetic and fascinating in the world. A dye can generally be described as a coloured substance that has an affinity to the substrate to which it is being applied [1]. Dyes are applied to various substrates (textiles, leather, paper, hair etc.) from liquid in which they are completely, or at least partially soluble [2], and may require a mordant to improve its fastness on the fibre [3].

The use of non-allergic, non-toxic and eco-friendly natural dyes has become a matter of significant importance due to the increased environmental awareness in order to avoid some hazardous synthetic dyes [4].

Dyes with azo bonds nitro- or amino-groups are carcinogenic, causing tumors of liver and urinary bladder in experimental animals. Reduction of azo dyes leads to formation of aromatic amines and several aromatic amines are known mutagens and carcinogens. Natural dyes offer an important alternative in these regards as
they are safer to use with no health hazards, have easy disposability, are biodegradable and can be used to make compost for agricultural purposes after they have been extracted [5].

Natural dyes are obtained from natural sources such as plants, insects and soil [6]. Several natural dyes are used for the study of histology, histochemistry and histopathology to define and examine bulk tissues such as muscle fiber, connective tissue, cell population of blood cells or organelles within individual cells. Staining procedures are required for detailed study and to prepare permanent preparations.

The most important and most used histological dye, haematoxylin is a natural dye produced from the logwood *Haematoxylon campechianum* and in combination with Eosin is used for the demonstration of general tissue structures [6].

*Curcuma longa*, commonly known as Turmeric is a tropical perennial herb belonging to the family Zingiberaceae. It is related to ginger and is grown throughout India, other parts of Asia and Africa [7]. Turmeric is commonly used as a spice and result in a bright yellow powder valued as a natural food dye [8, 9]. Turmeric makes a poor fabric dye, as it is not very light fast. *Curcuma longa* extract has been studied as a stain for collagen fibres and red blood cells [10].

This study was therefore designed to investigate the staining effect of *Curcuma longa* extract on the histomorphology of the testes.

**Material and Methods**

The leaves of *Curcuma longa* were purchased from a local market in Uyo local government area of Akwa Ibom State and authenticated at the Botany department of the University of Uyo, Nigeria.

**Preparation of extract**

The dried leaves of *Curcuma longa* were ground to a powder using the electric and manual grinding machine. A smooth consistency was ensured to attain good surface areas for light brown. The dry powder of the plant was weighed using an electric weighing scale. These were then soaked in ethanol for 24 hours undisturbed. The extraction was preceded using the Soxhlet extractor. The extract was then transferred to a rotatory evaporator.

There was further drying of the extract in a drying oven at 60°C. The temperature enabled the preservation of the active ingredients of the extract after it was obtained in powdered form.

**Preparation of sections**

The testes were carefully dissected out, trimmed of all fat and blotted dry to remove any blood. They were fixed in 10% formal saline. The fixed tissues were transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 hours, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicular to the long axis of the testes. The sections were designated “vertical sections”. Serial sections of 5 µm thick were obtained from a solid block of tissue using rotary microtome (SAKURA FINE TECH, Netherlands) and attached to sections and dried at 65°C for 45 min.

**Preparation of Curcuma longa staining solution and staining methods**

The ethanolic extract of *Curcuma longa* was dissolved in 1% acetic acid in 70%. It was used to stain testes sections at concentrations of 0.2 g/ml for 15 minutes. The sections were finally rinsed in water, dehydrated, cleared and mounted in a synthetic mountant.

**Preliminary phytochemical screening**

The extract of *Curcuma longa* was screened to determine the presence of the following metabolites through preliminary phytochemical screening. Alkaloids were detected using the Dragendoff’s reagent, Mayer’s reagent, Wagner’s reagent and tannic acid. Flavonoids were determined by the ferric chloride test, lead acetate test, sodium hydroxide test and ethyl acetate test. Tannin detection was by ferric chloride test and bromine water test, while phlobotannins was with hydrochloric acid. Saponin was determined with the froth tests and haemolytic test.
Results

Photomicrograph of testes stained with Curcuma Longa dissolved in 1% acetic acid in 70% ethanol at concentration of 0.2 g/ml for 15 minutes is shown in Fig. 1.

The Curcuma longa dye distinctly stained the seminiferous epithelium and interstitium yellow (Fig. 1 and Fig. 2). Curcuma longa provided a good counter stain for Haematoxylin, taking up the acidic staining characteristics with Haematoxylin staining the basic staining characteristics. Phytochemical screening revealed the presence of saponins, alkaloids, tannins and flavonoids.

Discussion

The increasing demand for material of natural origin is because of the toxic nature of many of the synthetic dyes; and the natural dyes are becoming widely recognized throughout the world [11]. The present scenario is focused more towards the utilization of the vast diversity of natural resources of colour pigments in place of their synthetic counterparts [12].

The dyeing of tissues is dependent on binding forces or link to the tissue; or they will simply be rinsed out of the tissue when the section is washed in another reagent. Ionic bonding involves electrostatic attraction between oppositely charged ions. It is the single most important form of bonding in most histological staining. Selectivity of staining depends on a sufficiently low dye concentration, on the time of action on the solvent, its aqueous or alcoholic nature and its pH.

A counter stain is a stain with colour contrasting to the principal stain, making the stained structure more easily visible. It is the application to the original stain, usually nuclear, of one or more dyes that by contrast will bring out heavy counter-stain is to be avoided least it mask the nuclear stain. It can be done either by using dilute stain or cutting down the staining time. Some counter-stains which are acidic may lighten or remove the nuclear stains. From the results obtained from this study, Curcuma longa can be used as a counter stain for Haematoxylin in lieu of Eosin.

Phytochemical screening of the dye confirmed the presence of saponins, tannins, flavonoids and alkaloids. Among these compounds, tannins and flavonoids are the substances which can give the colour. Tannins are the most important ingredients which are necessary for dyeing. Flavonoids are primarily recognized as the pigments responsible for the autumnal burst of hues and the many shades of yellow, orange, and red in flowers and food. 90% of all yellow dyes are flavonoids. The fastness of these yellow dyes is greatly affected by the mordant and the photosensitivity of the chromophores [13]. Saponins are known to reduce surface tension and this property also enhances staining [14].

In conclusion, Curcuma longa has great potential for use as a counter stain for Haematoxylin in lieu of Eosin.
References

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