International Journal of Basic, Applied and Innovative Research
IJBAIR, 2012, 1(4): 151 - 154
www.antrescentpub.com
ASN-PH-020919
ISSN: 2315-5388

RESEARCH PAPER

HISTOLOGICAL STUDY ON THE STAINING POTENTIALS OF AQUEOUS EXTRACT *OF CERATONIA SILIQUA* BARK

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Received: 17th August, 2012 **Accepted: 20th December, 2012** **Published: 31st December, 2012**

ABSTRACT

This study was designed to determine the staining potentials of aqueous extract of *Ceratonia Siliqua* bark adapted for the first time as a counter stain in Haematoxylin and Eosin staining reaction. The staining solution was used to stain liver, lung and kidney sections. Phytochemical screening of the extract revealed the presence of tannin (hennatonic acid or Lawsone), while results of the staining reactions showed that *Ceratonia siliqua* at a low pH, has intense staining qualities, confirming further that the effectiveness of a stain is influenced by the pH of its environment.

Key words: Ceratonia siliqua, Extract, Haematoxylin and Eosin, Counterstain, General tissue structure.

INTRODUCTION

Ceratonia siliqua, commonly known as the Carob tree and St. John's-bread, is a species of flowering evergreen shrub or tree in the pea family, *Fabaceae*. The tree grows up to 10 metres (33 ft) tall. It is native to the Mediterranean region including Southern Europe, Northern Africa, the larger Mediterranean islands, the Levant and Middle-East of Western Asia into Iran and the Canary Islands and Macaronesia (Battle and Tous, 1997). It is widely cultivated for its edible legume beans, and as an ornamental tree in gardens, it has a thick trunk with brown rough bark and sturdy branches (Missbah *et al.*, 1996). The seeds, also known as *locust beans*, are used as animal feed. They are also the source of locust bean gum, a thickening agent used in numerous processed foods (Turnbull *et al.*, 2006). The fruit contains leucodelphinidin, a colourless chemical compound, tannins (in leaves and bark), galactan, pentosan, (in seeds), mucilage and starch (Turnbull *et al.*, 2006). Extracts of leaves and bark have been recommended for the treatment of syphilis and venereal diseases, and seems to have a soothing effect on epilepsy (Turnbull *et al.*, 2006).

On the other hand, a dye is a coloured substance that has an affinity to the substrate to which it is being applied (Zollinger, 2003). The greatest source of dyes has been from the plant kingdom, notably roots, berries, bark, leaves and wood, but only a few have ever been used on a commercial scale. There are two types of dyes: natural dyes- obtained from natural sources, and synthetic dyes -produced through chemical reactions (Carleton *et al.*, 1976). Several natural dyes are used in histology, histochemistry, and histopathology. In fact the most important and commonly used histological dyes, is haematoxylin -a natural dye produced from the logwood, and Haematoxylon campechianum with Eosin (in combination), are used for the demonstration of general tissue structures (Avwioro, 2002).

However, some synthetic dyes have been known to cause some hazards to human and animal health and have therefore been withdrawn. Also, synthetic dyes are increasingly becoming expensive and are no longer within the reach of many small laboratories in developing countries. In addition, some synthetic dyes are not environmentally friendly (Sewekow, 1988; Eom *et al.,* 2001). It is for these reasons that an alternatively cheaper and bio-friendly natural dye extracted from *Ceratonia siliqua*, is been sort as a potential natural histological dye.

MATERIALS AND METHODS

Substance collection: Fresh bark of *Ceratonia siliqua* were collected from one of the carob trees in Otukpo, Otukpo Local Government Area of Benue State, Nigeria, and authenticated by a botanist in the department of botany, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

Substance preparation: They were rinsed in several changes of distilled water and drained. The bark were cut into tiny bits of about 1cm in diameter and dried in an open air for 72 hours. About 3 kg of the bark was extracted by boiling with 1 liter of water and allowed to stand for 24h. The extract was filtered three times using Whatman's Number one filter paper.

Preparation of sections: 3 human tissues, 3mm thick, were obtained from the liver, lungs and kidney at post mortem. They were fixed in 10% formol saline for 24hrs and processed for paraffin wax embedding with the automatic tissue processor (SAKURA FINETECH, Netherlands) by dehydrating through 70%, 90%, 95% and two changes of absolute ethanol for 90 min each. Clearing was achieved through changes of xylene twice for two hours each, infiltrating through two changes of paraffin wax at 70°C and embedded in paraffin wax. Sections were cut at 3µm with the rotary microtome (SAKURA FINE TECH, Netherlands) and attached to slides and dried at 65°C for 45 min.

Staining Methods: Sections were dewaxed in 2 changes of xylene for 2 minutes each, hydrated through graded solutions of 100, 95% and 70% alcohol, and then rinsed with water. It was stained with Harris haematoxylin for 5 minutes, rinsed in water and differentiated in 1% acid alcohol for 2 to 60 seconds. The sections were blued in running tap water and later stained with *Ceratonia siliqua's* extract for 2-60 minutes. Sections were finally rinsed in water, dehydrated in grades of alcohol (70%, 80%, 95% and absolute), cleared in xylene, air-dried and mounted with dibuthylphthalate propylene xylene (DPX).

RESULTS

Extraction was completed within 24h. This gave a reddish brown colour. The sections were compared with haematoxylin and eosin technique. *Ceratonia Siliqua* bark extract stained collagen fibres, red blood cells and muscle fibres light-brown within 15 min. Haematoxylin and Eosin (H&E) stained tissue sections presented normal cytoarchitectural features characteristic for H & E stained tissues, while haematoxylin and *Ceratonia Siliqua's* bark extract (H& *Ceratonia Siliqua's* bark extract) stained tissue sections presented poor cytoarchitectural distinctions (see figures 1A & B, 2A & B, and 3A & B).

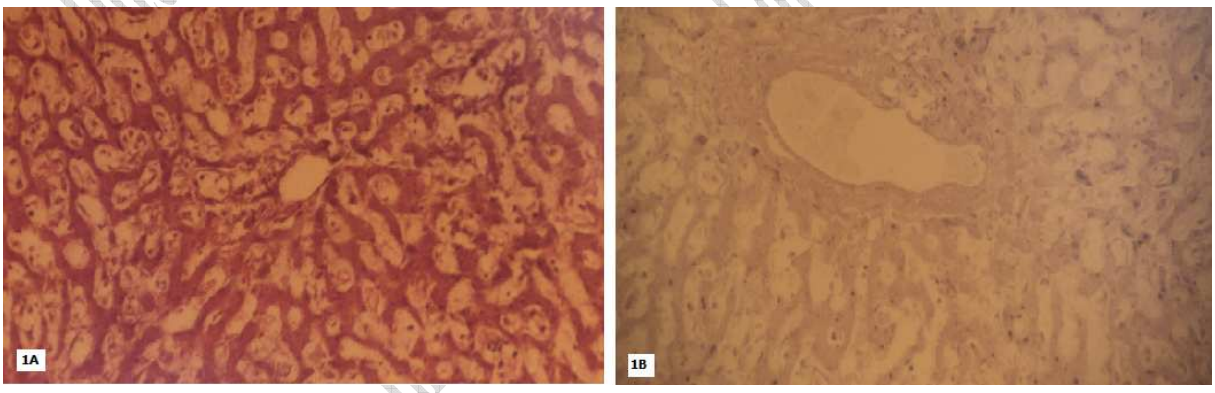

Figure 1(A and B): Control section of human liver (H&E x100) showing normal characteristics of Haematoxylin and Eosin stain (3A), and a section of human liver (H & *Ceratonia Siliqua's* bark extract x100) showing poor cytoarchitectural distinction (3B).

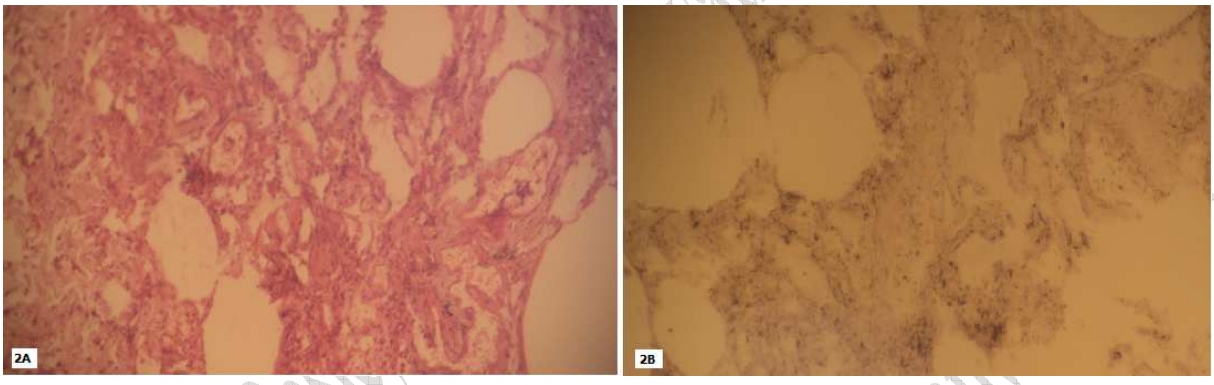

Figure 2 (A&B): Control section of Human Lung (H&E x100) showing normal tissue architecture (3A) and a section of human lungs (H & *Cetatonia siliqua's* bark extract x100) showing poor cytoarchitectural distinction (3B).

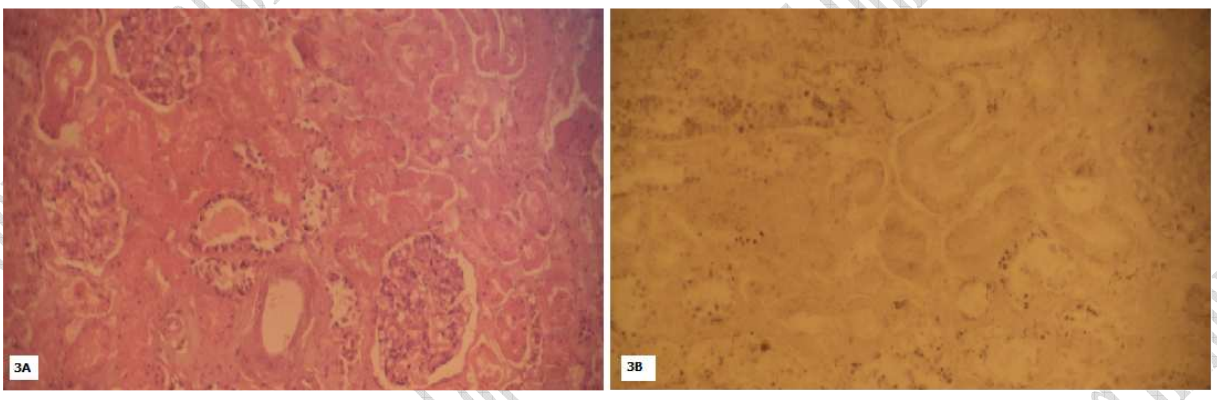

Figure 3 (A&B): Control section of human Kidney (H&E x100) showing normal characteristics of Haematoxylin and Eosin stain (3A) and a section of human Kidney (H& *Cetatonia siliqua's* bark extract x100 showing poor cytoarchitectural distinction (3B)

DISCUSSION

Cellular structures are selectively stained by various natural and synthetic dyes. Some requires a combination of stains to demonstrate the presence of tissue structures. Available literature reveals that the pH status (acidity or alkalinity) of a dye as well as the use of a mordant, affects the efficacy of some stains (Hoffman and Bauknecht, 1999). Our findings on *C. siliqua* bark extract show however, that the use of potassium aluminium alum as a mordant was not necessary because even simple aqueous solutions stained the tissues. This is unlike most dyes used in histochemistry, for example, haematoxylin, which is first oxidized to haematein and mordanted before it can be used as a stain for tissues. In fact, Elbadawi (1976) had stressed the need for the use of mordants in certain histochemical reactions and states that for the use of Verhoeff's iron haematoxylin as stain for elastic fibres, ferric chloride use as a mordant was necessary.

Furthermore, Hoffman and Bauknecht (1999) had observed that the ionic strength and pH of staining solutions often affect staining reactions. This however, was not observed with *C. siliqua* bark extract, as it stained at neutral, alkaline, and acidic media; though with a decrease in the quality of staining in the alkaline region. In fact, there are other natural and synthetic dyes, which do not require the addition of an acid or a base. Eosin, commonly used as a counterstain for haematoxylin, is a typical example of such a stain. Nevertheless, Vickerstaff (1954) in staining eosinophils with congo red, states that ionic strength influences the staining reaction between sulphuric acid groups of the dye and the basic groups of the eosinophil granules.

According to Horobin, (2002), acidic dyes are anionic and will stain cationic or basic groups in tissues such as amino groups. Such dyes are mostly used to stain proteins in the cytoplasm and connective tissues. On the other hand, basic dyes are cationic and stain anionic or acidic materials such as carboxylates, sulphates (many complex

carbohydrates are sulphated) and phosphates (particularly the phosphates in nucleic acids). Such basic dyes are used as nuclear stains and stains cytoplasmic carboxyl groups.

From the foregoing therefore, it is obvious that *C. siliqua* as a dye, can be said to posses some histological staining qualities and can therefore, be useful in histopathological investigations.

ACKNOWLEDGEMENT

We acknowledged the priceless support of all those (too numerous to mention), that contributed towards the success of this study.

REFERENCES

Avwioro, O.G. (2002): Histochemistry and tissue pathology. 1st ed. Claverianun press Nigeria. 134-213.

Batlle, I. and Tous, J. (1997). Carob tree. *Ceratonia siliqua* L. In: International Plant Genetic Resources Institute (IPGRI), Promoting the conservation and use of underutilized and neglected crops. (Prom Underused Crops) 17:1–92.

Carleton HM, Drumy RAB, Wallington EA, Cameron R. (1976). Histological technique. 4th edition. Oxford University Press. Lond. 108nnnnn.

Elbadawi, A. (1976) .Hexachrome modification of Movat's stain. *Stain Technol*. 51: 249 – 253.

Eom, S., Shin, D., Yoon, K. (2001). Improving the dyeability of natural colourants on cotton by cationization. *Ind J Fibre Text. Res.*; 26: 425-431.

Hoffman, E.M. And Bauknecht, N. (1999). A dye binding assay for the quantification of solution and cell-bound acidic polysaccharides produced by red algae. *Anal. Biochem.* 267 (2): 245 – 251.

Horobin, R.W. (2002) Theory of staining and its practical implications. In Theory and Practice of Histological Techniques, 5th Edn (Eds J.D. Bancroft and M. Gamble). Edinburgh: Churhill Livingstone.

Missbah EM., Aujjar, N., Belabed, A., Dessaux, Y., Filali-Maltouf, A. (1996). Characterization of rhizobia isolated from Carob tree (Ceratonia siliqua). *Journal of Applied Microbiology;* 80 (2): 165–73.

Sewekow, U. (1988). Natural dyes- an alternative to synthetic dyes. *Melliand Textilberichte*. 69 (4), 145-48.

Turnbull, L.A., Santamaria, L., Martorell, T., Rallo, J. and Hector, A. (2006): Seed size variability: from carob to carats. *Biology Letters; 2 (3):* 397–400.

Vickerstaff, T. (1954). The physical chemistry of dyeing, Olive and Boyd, London. p. 352.

Zollinger, H. (2003). Color Chemistry. Synthesis, Properties and Applications of Organic Dyes and Pigments, 3rd ed. Weinheim: Wiley-VCH.

AUTHOR(S) CONTRIBUTION

All the authors in this article contributed one way or the other to the success of this study and in the presentation of this paper.