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A Bioprospective Study on Histological Staining Potential of *Beta Vulgaris* (Beetroot) Extract.

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ABSTRACT

The uses of eco-friendly and biodegradable materials have been a key agenda for global advocacy. We explored the histological staining effect of *Beta vulgaris* (beetroot) extract on selected tissues of Wistar rats. Four healthy rats were sacrificed under ether anaesthesia. The liver, lung and brain were harvested and fixed in 10% neutral formal saline. The tissues were manually processed and stained using various procedures as follows: Sections A (Haematoxylin and Eosin), B (Beetroots extract alone), C (Beetroots extract and Eosin), and D (Beetroots extract, Eosin and vinegar as mordant). The beetroots extract alone exhibited haematoxylin acidophilic- like effect on the selected tissues. The extract and eosin combined fairly stained the cellular features compared with the routine H and E stain. An improved effect was observed in sections stained with the extract, eosin and vinegar as (mordant). The extract showed specificity for the lungs tissue. Beetroot extract can be considered as an alternative for Haematoxylin in the routine H and E histological staining technique and may require mordant as stain enhancer for some tissues particularly for demonstrating the lungs.

Keywords: Dyes, Mordant, Histology, Beet extract, Lungs, Cerebral Cortex.

INTRODUCTION

The uses of eco-friendly and biodegradable materials have been a key agenda for global advocacy. This is necessitated by the continual depletion of the ecosystem by bioharzadious substances that adversely disturb the symbiotic relationship in the ecosystems (Pereira and Alves, 2012). Biomedical wastes products generated from chemical reagents contribute to the volume of hazardous environmental agents and the consequent impact on the ecological system. Most of the biomedical reagents used in hospital laboratories for histological, histopathological, biochemical and imaging techniques are hazardous to human health and environment upon exposure. In addition, the economic burden is high due to the expensive nature of routine laboratory reagents. These have stirred the search for natural materials that are ecofriendly, biodegradable, inexpensive and safe for routine commercial use in laboratories.

Hence, the use of natural material is experiencing resurgence in biomedical research in recent times. We considered the search for alternative histological stain that is biosafe, inexpensive and ecofriendly such as natural dyes. There are evidences where people have dye fabrics and woods using locally available materials which produce brilliant permanent colours (Ashis and Adwaita, 2011). Majority of these natural dyes are vegetable dye obtained from plant parts such as the roots, barks, berries, leaves and fruits (Grubben and Deton, 2004) which have long history of been used traditionally as dye for fabrics and woods. Examples are cochineal and leg wood (*haematoxylum campecianum)*, hematoxylin has being implored in preclinical and clinical histological and histopathological routine procedures as stain for tissues to aid disease diagnoses. The hematoxylin is an acidic stain that stains the nuclei and counter stained with eosin a

basic stain which stains the cytoplasm.

Beet root is a purple root vegetable plant cultivated mostly in the North-Eastern parts of Nigeria particularly in Plateau State and transported to other parts of the country. Beet root is cherished for its nutritional and medicinal values thus it is used in cuisines, salads and juices. In addition, it is affordable and readily available in vegetable stores, farmers and public markets within the country. Beetroot (*Beta vulgaris)* has a long history as dye for fabrics and woods, it impacts purple to red colour. The essential processes of dyeing and staining tissues share certain similarities, in the dyeing process, the dye is placed in a pot of water then the fabric to be dyed is added to the pot and heated with intermittent stirring until the colour is transferred (Seong-il et al. 2001). While, histological staining technique involves stepwise tissue processing stages undertaken in different media and timed to allow tissue/cell components to acquire the specific colour (s). Like some histological stains natural dyes may also require mordants such as salts, alum, vinegar and ammonia to bind the dye to the textile. The beetroot dying process may require alum or vinegar mordant to enhance the colour intensity and durability, however this depend on the material to be dyed (Seong-il et al. 2001).

Our study is a bioprospective study that x-rays the histological staining potential of beetroot extract on selected tissues. It also compared the staining effect to hematoxylin and eosin and examines the role of vinegar (mordant) on the staining effect of beetroot. This provides an alternative stain and opens up a window for research collaboration on natural dye prospects for tissue stains and economically creates entrepreneurship opportunity for the scientist.

MATERIALS AND METHODS

Collection and Preparation of Beetroot dye

Fresh beetroots were bought from commercial vegetable dealer in Enugu State, Nigeria. The beets were washed in water to remove impurities ad cut into smaller cubes followed by blanching. The water blanching method described by (Karla, 1990) was adopted but modified; blanching was to inactivate the enzymes and retains the purple colour of beetroots. The cubes to be blanched were loosely tied, 50grams of the beets in 100ml of water in a transparent sieving material and were dipped in hot water at 80°C for 3-5 minutes. Immediately after the blanching the cubes were held under running tap water to prevent further cooking. It was then filtered using the whatman filter paper No.1 to obtain purple coloured dye. The filtrate obtained was oven-dried to get rid of some residual water to form a concentrate.

Experimental animals and Ethical approval:

Four (4) adult Wistar rats of both sexes were bought from animal breeder in Nsukka, Nigeria and transferred to the animal facility of the College of Medicine, Enugu State University of Sciences and Technology (ESUT) Nigeria. The experimental protocol was approved by the Departmental Research Ethics Committee. All protocols were carried out in strict accordance with the guidelines for the care and use of animals for research.

Animal Sacrifice and Tissue Collection

The rats were sacrificed under ether anaesthesia, the thorax; abdomen and brain were dissected to harvest the lungs, liver and cerebrum. The tissues were fixed immediately in 10% formal saline for 72hrs and standard manual tissue processing techniques was used. Sections were cut at 10µm thickness using a rotary microtome. The tissue sections were stained variously as follows: (i) alum Haematoxylin and Eosin, which served as the control. (ii) beets extract and eosin (iii), beets extract only and (iv) beets extract and eosin with vinegar mordant. The stained slides of all groups were viewed and analyzed using a light microscope for histological similarities and differences, after which the slides were captured with Amscope light microscope.

Staining Procedures

Hematoxylin and Eosin (H and E) Technique (control)

The tissue sections were dewaxed in xylene and hydrated through descending grades of (95%, 80%, 70%) alcohol. Sections were rinsed in water briefly and stained in Harris Haematoxylin for 4mins. The sections were then brought to water to remove excess stain and differentiated in 1% acid alcohol for 1min.

Sections were also blued in running tap water for 15mins and counter stained with Eosin for 4mins. Sections were rinsed finally in water, dehydrated in ascending grades of absolute alcohol (70%, 80%, and 90%), cleared in xylene cover slips were mounted using DPX (Distyrene, Plasticizer and Xylene). Prepared slides were allowed to air dry and examined under a light microscope and photomicrographs were captured.

Beet Extract and Eosin (B and E)

The tissue sections were dewaxed in xylene and hydrated through descending grades of (95%, 80%, 70%) alcohol. Sections were rinsed in water briefly and covered with Beetroot extract for 30mis -1hrs. Sections were washed in water and counter stained with Eosin for 2mins. Sections were rinsed finally in water, cleared in xylene and cover slips were mounted using DPX. The prepared slides were allowed to air dry and examined under a light microscope and photomicrographs were captured.

Beet Extract Only

The tissue sections were dewaxed in xylene and hydrated through descending grades of (95%, 80%, 70%) alcohol. Sections were rinsed in vinegar briefly and covered with Beetroot extract for 30 mins -1hrs. Sections were then washed in Vinegar, rinsed finally in water and cleared in xylene. Cover slips were mounted using DPX. The prepared slides were allowed to air dry and examined under a light microscope and photomicrographs were captured.

Eosin and Beet Extract in Vinegar

The tissue sections were dewaxed in xylene and were hydrated through descending grades of (95%, 80%, 70%) alcohol. Sections were rinsed in vinegar briefly and covered with Beetroot extract for 30mins-1hrs. Sections were washed in Vinegar and counter stained with Eosin for 2mins. Sections were rinsed finally in water, cleared in xylene and cover slips were mounted using DPX. The prepared slides were allowed to air dry and examined under a light microscope and photomicrographs were captured.

RESULT AND DISCUSSION

Majority of histological stains exhibit an ionic bond between the tissue section and the stain. The binding principle of tissue to stain depends on the nature of bond formed between the dye and the tissue components. Besides, the bond formation other factors also contribute to the selectivity and/or specificity of a dye/stain to demonstrate a tissue component. These factors include: dye concentration, time of action on the solvent, its aqueous or alcoholic nature and its pH (Bancroft and Cook, 2013).

Haematoxylin is a basic dye which stains the nucleus and some parts of the cytoplasm containing the nucleic acid or acidic structures bluish purple or black in some instance. In this study, it was observed that the beetroot extract and haematoxylin have similar staining effects by staining tissue component purple to black (figure B). This suggests that beetroot extract exhibited hematoxylin like staining effect. On the contrary, eosin an acidic dye will stain basic structures deep pink colour. The exceptions to these are neutral cellular and extracellular components that take up neither of these stains and appear relatively clear (Anneh et al. 2006). This implies that the extracts having exhibited hematoxylin property can be used in combination with eosin.

In addition, the beetroot (*Beta vulgaris*) has rich active compounds including and not limited to such as Carotenoids, glycine, saponins, betacyanins, folates, betanin, polyphenols and flavonoids. Its dyeing property has been attributed to the presence of betanin (Tom, et al. 2015). Although other factors like the degree of acidity, alkalinity and mordants differentially determine the staining property (Elbadawi, 1976). Hence, the need for mordants in certain histochemical reactions are sometime required to binds the stain onto tissue sections by forming a complex and to enhance the retention of the stain to the tissue (Lynch et al. 1969; Bancroft and Cook, 2013). In this study, when eosin was used as counter stain for beetroot extract and vinegar (mordant), the tissue sections revealed comparable results to haematoxylin and eosin stain in the selected tissues (figures A and D). These stained sections were also better than beet and eosin (B&E) combination alone. It thus, suggests that mordant (vinegar) may be required

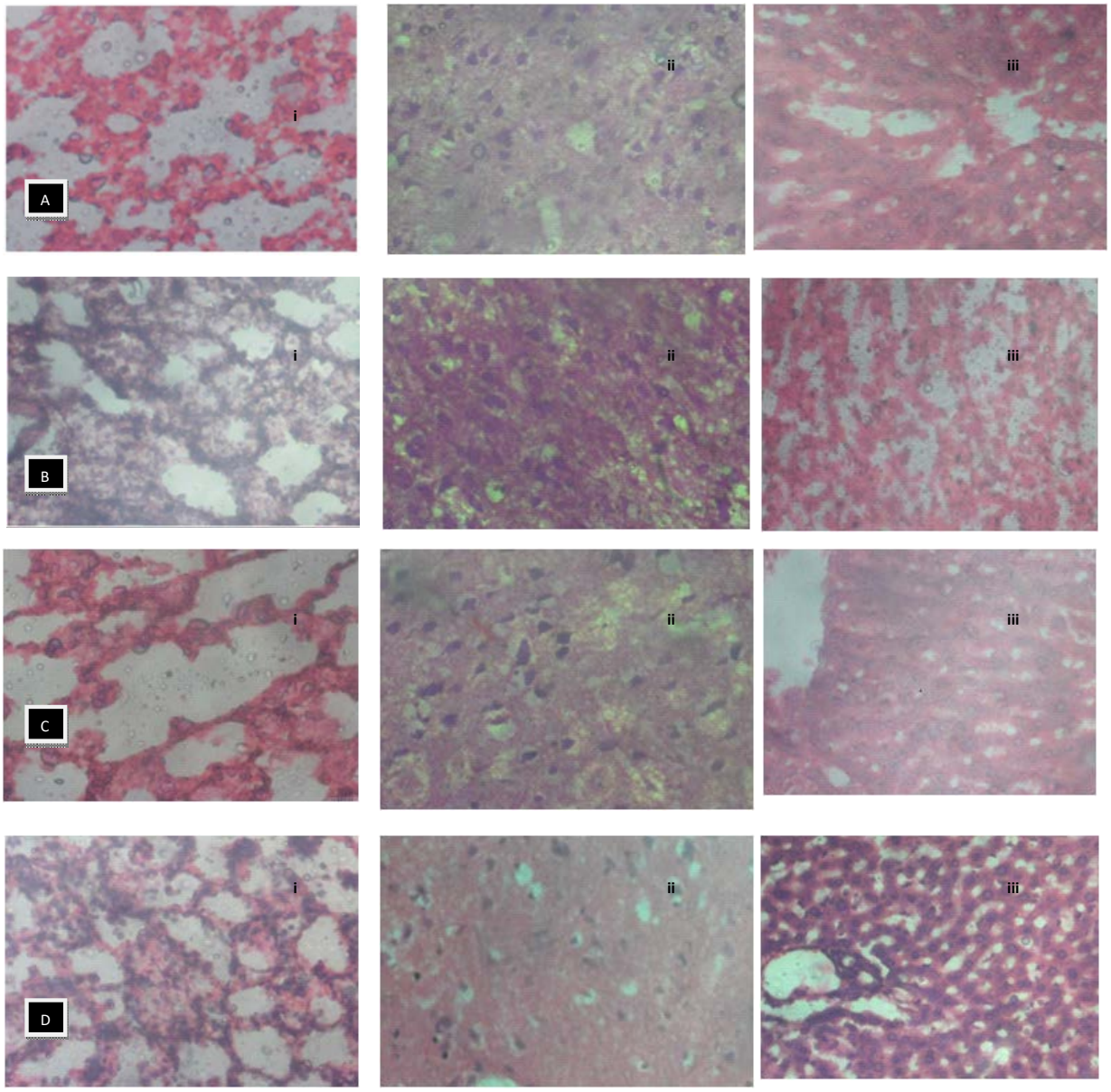

Figure I: Sections of tissues i= lungs, ii =cerebral cortex, iii =liver) stained: A (Hematoxylin and Eosin), B (Beets extract alone), C (Beets extract & Eosin), D(Beets extract, eosin and vinegar) x160

when staining tissues with beets extract and eosin for enhanced staining effect.

Amongst the selected tissues the lungs histoarchtecture was better stained with the various beetroot extract techniques than the liver and cerebral cortex (figure B, C and D i). This is a display of stain selectivity for a tissue but, the H and E clearly distinguished the cytoplasm and nuclei particularly in the combined stains. This effect was attributed to non blueing of tissue in acid alcohol which will be taken into consideration in further studies.

CONCLUSION

In conclusion, beetroot extract has haematoxylin- like staining effect on the lungs, liver and cerebral cortex, it could serve as a substitute for hematoxylin and counter stained with eosin. The use of mordant and blueing agents might be required depending on the procedure and tissue to give a comparable effect with hematoxylin and eosin, and selective or

specific stain for the demonstration of the lungs.

Conflict of Interests.

Authors have declared that they have no competing interest.

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