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Natural Dyes an alternative to conventional histological staining techniques: Investigating aqueous Avocado seed extract dye

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ABSTRACT

The global concern about the use of eco-friendly and biodegradable materials in tissue staining has borne laudable interest in the use of natural dyes as suitable alternatives to synthetic dyes. Plants like avocados serve as dyeing agents for fabrics, but little is known about their potential as a histological stain. This study focused on investigating the staining properties of the aqueous avocado seed extract on histological sections of the spleen, testis, skin, and pancreas. A total of five fresh avocado seeds were chopped into bits and simmered in water to extract their deep maroon dye. Tissue sections of the spleen, testis, skin, and pancreas of 4 adult Wistar rats aged 12 weeks and weighing 180–250 g were used. They were grouped into four, labeled A to D, with each group containing 4 specimens (1-Spleen, 2-Testis, 3-Skin, and 4-Pancreas). Group A (control) was stained using the Haematoxylin and Eosin technique; group B was stained using Haematoxylin and Avocado seed extract; group C was stained using Avocado seed extract and Eosin, while group D was stained using Avocado seed extract only. Findings revealed blue-black nuclei and pink cytoplasm (group A); blue-black nuclei and brownish but unclear cytoplasm (group B); pinkish cytoplasm with no nuclei seen (group C); brownish cytoplasm with unclear features (group D); only the testis demonstrated remarkable features. This study demonstrated the properties of avocado seed extract as an acid dye capable of staining the cytoplasm, and may have a better inclination towards staining testicular cytoplasm.

Keywords: Avocado Seed, Haematoxylin and Eosin, Natural Dyes, Testicular tissue.

INTRODUCTION

According to the Collins Dictionary of Medicine, Staining is defined as the use of selected dyes - a natural or synthetic colored substance that has an affinity to the substrate to which it is being applied; to color biological specimens such as cells, cell products, and thin slices of tissues or micro-organisms, to assist in examination and identification under the microscope[1]. Dyes are known to be primarily from two sources; either natural sources or man-made (synthetic) sources. Natural dyes come from plants, invertebrates, or minerals, while synthetic dyes are man-made. Natural dyes can also serve as colorants for foods and drugs[2]. The bulk of natural dyes come from biological sources like fungi and lichens as well as plant sources like roots, berries, fruits, seeds, flowers, leaves, and stems. Under this, one can discover plants that can be utilized for histological staining, such as avocados, hibiscus flowers, beetroot, and others. Numerous classifications are possible for natural dyes[3]. The oldest classification used the botanical names of plants, their origin, their hue, their chemical makeup, and their class of application[3, 4]. Natural dyes categorized according to their chemical makeup may include flavonoids, which yield a yellow-colored dye, and anthocyanidins, which give a direct orange-colored dye[5]. Avocado seed dye is an example of this classification, and in addition to containing mainly flavonoids and anthocyanins, it has traces of carotenoids and chlorophylls[6-8].

Long believed to have originated in south-central Mexico, the avocado (*Persea americana* mill) is a fruit [9]. It also goes by the name "Alligator Pear," which is the botanical name for a huge fruit with a large seed [10]. They feature a fleshy, green-skinned body that can be spherical, pear-shaped, or even black in some cases. Avocado is commonly utilized for its flesh, while its seed is considered waste [11]. The crushed avocado seed has the potential to serve as a natural alternative for histological staining, as it produces a natural orange/deep maroon dye when extracted with water [12]. Avocado seed and peel can produce dyes that can be used to color hair and clothing, and some consider it a substitute for natural food dyes [11, 13, 14]. Given that it produces a color that is potent enough to be used in histology laboratories as a stain or dye, no conclusive study has been carried out to elucidate this hypothesis.

Although synthetic dyes are more effective, there have been reports that they are harmful to human health, which has reduced their use [15, 16]. Safranin-O and methylene blue (MB), two synthetic dyes frequently employed as tissue dyes, are relatively simple to utilize in microscope examinations but pose serious detrimental effects on health [17]. Safranin-O is one of the cationic dyes whose waste can create dangerous contamination for pharmaceutical and textile manufacturers. In addition, safranin-O dyes have negative effects on human health, such as skin allergies [18]. It is pertinent to note that MB, worse than Safranin-O, contributes to anemia, dizziness, fever, headaches, and cardiovascular disorders [19]. Utilizing natural dyes is an alternate approach to solving this issue as they are more environmentally friendly, non-toxic, non-carcinogenic, and biodegradable [17, 20]. The use of natural dyes has once again become more significant as a result of the widespread concern about the usage of eco-friendly and biodegradable products, therefore prompting the use of less expensive, naturally occurring plant dyes as a substitute for synthetic dyes [21].

Experimental Design

Since there are some plants, like avocados, that can act as coloring agents, it is necessary to determine whether or not their staining ability can be used in place of synthetic dyes in histological techniques.

MATERIALS AND METHODS

Preparation of Aqueous Avocado Seed Extract Dye

The materials used for the aqueous avocado seed extract (AASE) in this study consisted of five (5) fresh avocados (*Persea americana*) procured from a fruit shop at Ogbete Main Market, Enugu-North LGA in Enugu State, Nigeria. The seeds from the avocado fruits were harvested and the outside skin peeled. The peeled seeds were ground into bits of less than 0.2 x 0.2 x 0.2 cm, and collected into a jar containing 1300 cubic centimeters of water. The jar with its content was simmered in a water bath at 60ºC for approximately 45 minutes while constantly stirring. The jar of AASE was cooled at room temperature for 24 hours. The final color of AASE changed from pink to deep maroon after 24 hours.

Procurement of Experimental Animals and Tissue Processing

A total of four (4) adult male Wistar rats, aged 12 weeks and weighing 180 – 250 g procured from the animal house of the Department of Anatomy, Faculty of Basic Medical Sciences, Enugu State University of Science and Technology, and acclimatized for two weeks. They were housed in a well cross-ventilated cage system and allowed easy access to food and water, under standard laboratory conditions and handling in the animal house facility. The animals were euthanized using barbiturate overdose at 100 mg/kg. The spleen, testis, skin, and pancreas were carefully dissected from each of the rats and fixed in 10% buffered formal saline before processing.

Table 1: The Experimental design

Experimental Group	Specimen	Histological Technique
Group A (Normal control)	Pancreas Skin Spleen Testis	Stained using Hematoxylin & Eosin technique only
Group B	Pancreas Skin Spleen Testis	Stained using Hematoxylin & Aqueous Avocado Seed Extract
Group C	Pancreas Skin Spleen	Stained using Aqueous Avocado Seed Extract only

	Testis	
Group D	Pancreas Skin Spleen Testis	Stained using Aqueous Avocado Seed Extract & Eosin

RESULTS AND DISCUSSION

Histological Findings for Tissues Stained with Hematoxylin and Eosin

As shown in Figure 1 series where sections of tissues from the pancreas, skin, spleen, and testes were stained with hematoxylin and eosin techniques, the normal routine histoarchitectural features of these tissues were observed. Hematoxylin, a basic dye stained the acidic component (the nuclei) of the cells blue-black in color. In contrast, eosin as an acidic dye stained the basic component (cytoplasmic structures) of the cell pink. This is in agreement with the works of Keirman [22] and Llewellyn [23] who acknowledged that staining with hematoxylin and eosin is one of the most preferred tissue staining techniques for routine histology due to the distinctive staining characteristics of these dyes.

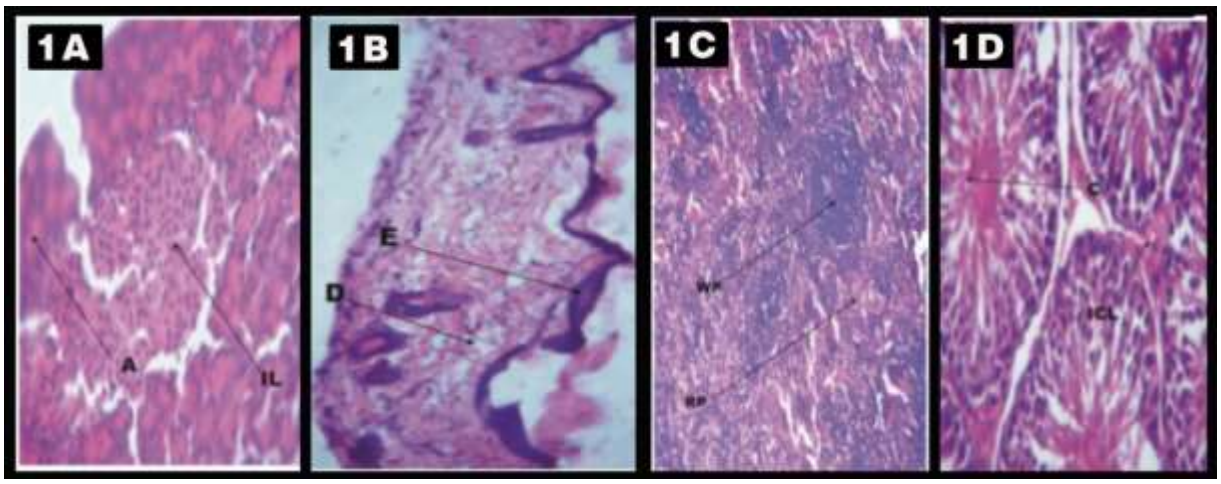

Figure 1: Tissues in group A, 1A-1D (Pancreas, Skin, Spleen, and Testis) stained using the H&E technique only demonstrated blue-black nuclear staining and pink cytoplasmic staining. **MG x100.** In Figure 1A; the pancreatic tissue with acini cell (A) stained blue-black, with the islet of the Langerhans (IL) and the cytoplasmic components stained pink. Figure 1B showed skin tissue with the epidermal layer (E) stained blue-black while the dermal layer (D) stained pink. Figure 1C demonstrates the spleen tissue with white pulp (WP) stained blue-black and red pulp (RP) stained pink. Figure 1D displays the testicular tissue with the Sertoli cells and interstitial cells of Leydig (ICL) stained blue-black and the cytoplasm (C) stained pink.

Histoarchitecture of tissues stained with Haematoxylin and Avocado Seed Extract

In histopathology, hematoxylin is the most commonly used dye obtained from a South African tree known as logwood (*Hematoxylium campechianum*. *L*) [24]. It has little or no staining capacity until oxidized to hematein. Therefore, hematoxylin, as a natural histochemical and histological dye requires a mordant before staining [25]. It is a basic dye and thus, stains the acidic component (the nuclei) of a cell blue-black. This is the case as seen in the histological sections of the Figure 2 series where the nucleus of the cells in the pancreatic, skin, splenic, and testicular tissues were stained blue-black with a clear cytoplasm. This also confirms that hematoxylin has no cytoplasmic staining effects.

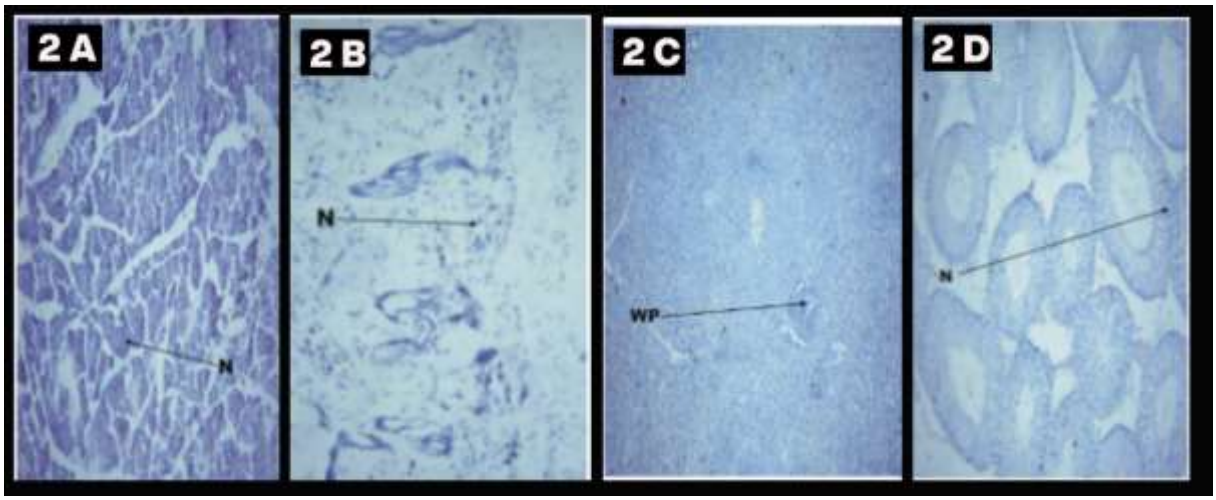

Figure 2: Tissues in group B, 2A-2D (Pancreas, Skin, Spleen, and Testis) stained using the H & E technique and Aqueous Avocado Seed Extract, demonstrated blue-black nuclear staining and brownish but unclear cytoplasmic features. **MG x100.** In this group, only the nucleus of the tissues was stained and they appeared blue-black.

Histoarchitecture of tissues stained with Avocado Seed Extract only

Photomicrograph of pancreatic, skin, splenic, and testicular section stained with avocado seed dye appear brownish with no nuclei as seen in the Figure 3 series. The absence of nuclear representation suggests that this dye may be acidic and consequentially will have no nuclear staining capacity. Figure 3D which was a testicular tissue stained with the avocado seed dye, showed a somewhat normal arrangement of the seminiferous tubules of the testes but with the total absence of the nuclear properties. This fact suggests that the dye has a cytoplasmic staining effect and agrees with the above suggestion of the dye being an acid dye. Although this cytoplasmic staining effect observed in the testicular tissue is poor, it agrees with the suggestion by Deepti [12], that the avocado seed may be a potential source of natural colorant.

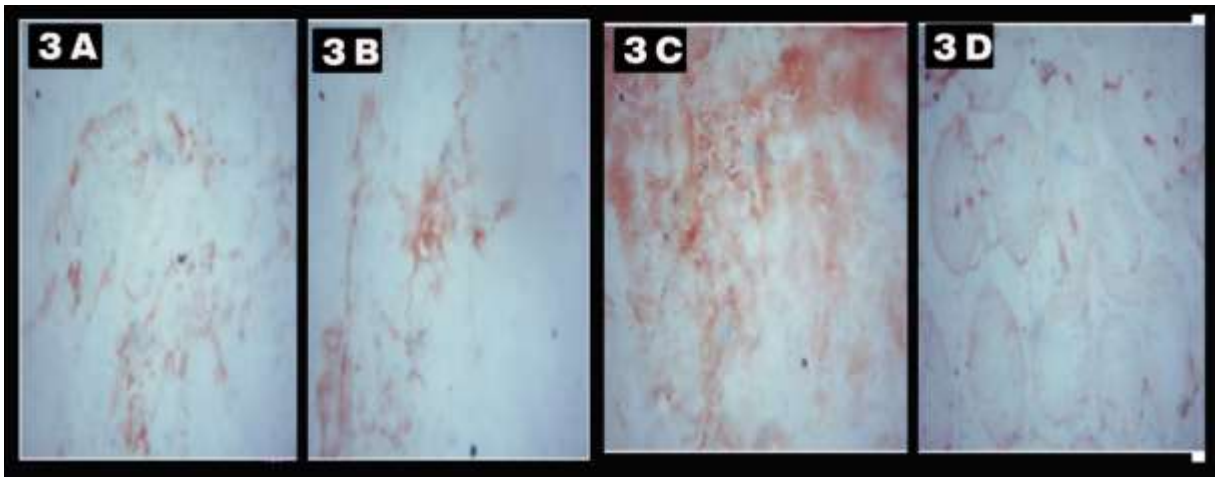

Figure 3: Tissues in group C, Figure 3A-3D (Pancreas, Skin, Spleen, and Testis) stained using Aqueous Avocado Seed Extract only, demonstrated brownish but unclear cytoplasmic features, only the testis (Figure 3D) demonstrated remarkable features. **MG x100.** In group C, only the cytoplasm in these photomicrographs stained brownish. Although not all tissues were stained clearly, the cytoplasmic components of the testis were delineated better than the rest of the tissues in this group. No nuclear was seen or demonstrated.

Histoarchitecture of tissues stained with Avocado Seed Extract and Eosin

The photomicrograph of pancreatic, skin, splenic, and testicular sections stained with avocado seed dye and eosin as seen in Figure 4 photomicrographic series all appeared eosinophilic (cytoplasmic) with no visible nuclear particle. This also confirms the fact that eosin is an acid dye that stains the basic component of the cells (cytoplasm) only. As no nucleus was visible, it also agrees with the above suggestion that the avocado seed dye has no nucleic staining property.

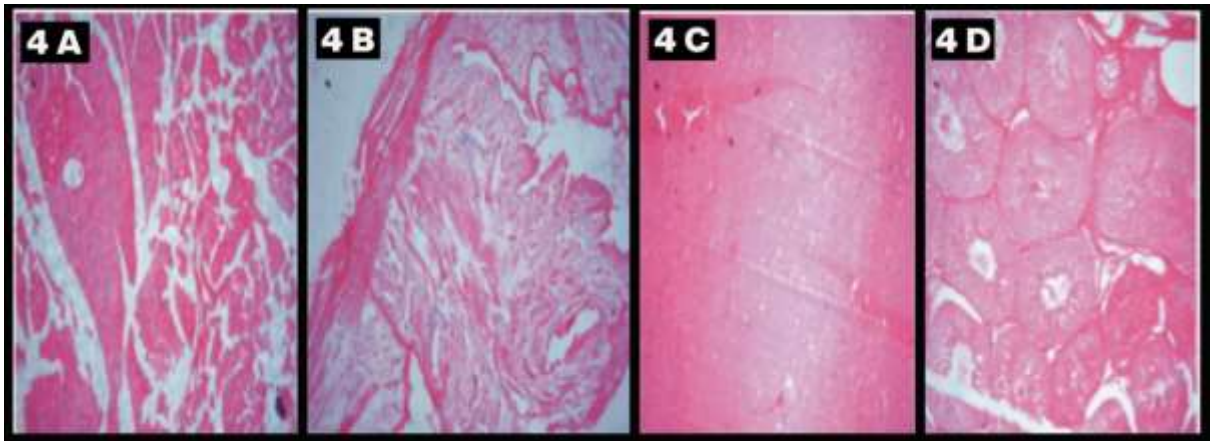

Figure 4: Tissues in group D, 4A-4D (Pancreas, Skin, Spleen, and Testis) stained using aqueous Avocado seed extract and eosin, demonstrated pinkish cytoplasmic staining with no nuclei seen. **MG x100.** Group D had only the cytoplasm demonstrating a pinkish stain attributed to the eosin dye. The photomicrograph of Figure 4D demonstrates testicular features but with no nuclei seen.

Cellular structures are selectively stained by various natural and synthetic dyes. Some require a combination of stains to demonstrate the presence of some of these tissue structures. Acidity, alkalinity, and mordant have been reported to affect some stains [26]. The findings from this study suggested that the Avocado Seed dye is an acid dye, and thus has a cytoplasmic staining effect with a brownish appearance thus, was incapable of staining the nuclei. It also suggests that this dye may have a better preference for staining testicular tissues.

The findings from this study also indicated that the use of vinegar as a mordant produced no significant effect on the staining quality of the Avocado Seed dye and eosin as all tissue sections stained with a combination of Avocado Seed dye, eosin, and vinegar as a mordant all appeared eosinophilic (cytoplasmic) with no visible nuclear particle, but since hematoxylin is a basic dye that stains the acidic components of tissues, it can serve as a counterstain for avocado dye in histological staining.

Conclusively, further studies should be carried out to back up the claims made by this study in higher concentrations of this dye and further mixtures of acidic and alkaline solutions. Furthermore, studies should also be carried out to assess the active phytochemical constituents responsible for the staining capacity of this plant extract.

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Competing Interests

The authors have declared that no conflicting interests exist.

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