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The Predicted Ability of Lawsonia Interims (Henna) to Stain Histological Tissue Sections and Malaria Blood Smears

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Abstract: *The microtome sections of the tissue biopsies are colorless, making it impossible to distinguish between the various tissue components identification and the study of their morphology are made possible and straightforward by staining the specimens with various colored dyes that have an affinity for particular tissue components. in order to investigate the hypothesis that Lawsonia inermis, often known as "henna" could serve as a good source of nuclear or cytoplasm staining. Fresh leaves of the henna tree were collected, thoroughly washed under running water, dried, and subjected to grinding with the aid of a mechanical grinder to form powder, sieved, and stored in a dry container 10g of henna powder was then dissolved in 100ml of absolute alcohol to give a concentration of 100mg plant product/ml of absolute alcohol, Then to the alcohol were added 100 ml of glycerin, 100 ml of distilled water, 10 ml of glacial acetic acid, and 15g of potassium alum the addition of sodium Iodate caused the henna solution to chemically ripen, ammonia, potassium alum, and acetic acid were added to an ethanolic henna solution to increase Lawsonia inermis' affinity to acidic structure nuclei, according to the general staining principle that only basic stains do color the acidic structure nucleus, the alkalinized solution showed improved staining of the acidic nucleus, henna also stained malaria parasite weakly brown at a concentration up to 1og/100 ml, indicating that the acidic nature of henna plant was probably responsible for their staining to the basic cellular component cytoplasm. The results of this study should serve as a foundation for further investigation and research into the potential utility of natural plant products as sources of histology dyes.*

Introduction

The scientific name for the tall shrub plant, Lawsonia Inermis (L. inermis), also known as henna, mehndi, or the mignonette tree, is Kingdom: Plantae, Division: Angiospermae, Class: Dicotyledoneae, Order: Myrtales, Family: Lythraceae, Genus: Lawsonia. L. inermis is the species [3]. A flowering plant with a 5-meter height, henna is native to subtropical and tropical regions of the world, including South Asia, Africa, Saharan Desert oasis, and even northern Australia.A tall shrub plant known as henna is scientifically known as Lawsonia Inermis (L. inermis) [1] or mignonette tree [2] is a member of the following families and orders: Myrtales, Family Lythraceae, Genus Lawsonia, Species L. inermis, Kingdom Plantae, Division Angiospermae, Class Dicotyledoneae[3] A flowering plant with a 5-meter height, henna is native to subtropical and tropical regions of the world, including South Asia, Africa, Saharan Desert oasis, and even northern Australia. Henna plant leaves are smooth, oval, whole, opposite, and sub-sessile [4] Leaves are 1-2 cm wide and 2-3 cm long [1] ,the greyish-brown bark of henna shrubs is heavily branching [5] Henna tattoos have been used in Sudan since ancient times as a skin ornament and part of wedding celebrations. However, Sudanese women don't typically apply henna on their hands or feet until they get married. The bride's female family members throw a gathering known as a "Henna day" before to the wedding .All tissues and the cells that make them up are typically transparent and colorless, making it difficult to discern between various structures under a traditional light microscope. Fortunately, the histological methods used to distinguish between tissues frequently result in two changes in the tissue: either a change in contrast or a change in color. The staining technique can be used to produce these effects. Specific and sensitive staining techniques are necessary for success. Ability to distinguish between different tissue components and color one or a few of them while leaving others uncolored is known as specificity or selectivity. After tissue processing, tissues taken from human or animal bodies are visible[6]. Essential oils, 1,4-naphtho-quinone, tannins, flavonoids, lipids, sugars, tri-acontyl tri-decanoate, mannitol, xanthones, coumarins (5-alkyloxy-7-hydroxy-coumarin), (2-3%) resins, (5-10%) tannic compounds, and up to 2% lawsone are all naturally occurring components of henna (2-hydroxy-1,4-naphtho-quinone). The primary natural dye present in lawsone as (1.0-1.4%) was glycosidic-bound to a significant extent, and it was released by enzymatic hydrolysis of the glycosidic hennosids and auto-oxidation of aglucons[7] Additionally present were coumarins (esculetin, fraxetin, scopletin), steroids (-sitosterol), 1,4-dihydroxy-naphthalene, 1,4-naphtho-quinone, 1,2-dihydroxy-glucoyloxy-naphthalene, and 2-hydroxy-1,4-diglucosyloxy-naphthalene[8]. ,contained a soluble matter tannin, gallic acid, glucose, mannitol, fat, resin and mucilage[9], were yield tannic acid and olive oil green resin, the un-saponified matter contained waxes and colouring matter[10],contained carbohydrates, proteins flavonoids, tannins and phenolic compounds, alkaloids, terpenoids, quinones, coumarins, xanthones and fatty acids. In addition, henna is reported to show some other properties including hypoglycemic [12], immunostimulant [10], hepatoprotective [11], anti-inflammatory [13], tuberculostatic [14], anti-cancer and antioxidant properties [15].Sudanese henna products are Zaharat Eldammar ,Bitelnile and Taj- Eldefafe, large quantities of henna are produced at home for the local markets and smaller quantities sold on the international markets .the demand of henna has certainly grown in Europe and north America over the past few years according to Sudan ministry of trade. This study sought to determine whether an ethanolic extract from Lawsonia inermis leaves could stain liver, spleen, and intestine sections as well as malaria blood smears.

Materials and methods

Preparation of plant materials

Fresh leaves of Henna Tree were collected and washed thoroughly under running tap water then dried and subjected to grinding with the aid of a mechanical grinder to form powder, sieved and stored in a dry container.

Preparation of the henna staining solution

10g of henna extracts was dissolved in 100ml of absolute alcohol to give a concentration of 100mg plant product/ml of absolute alcohol. Then Glycerin 100 ml, Distilled water 100 ml Glacial acetic acid 10 ml Potassium alum 15 g then stain was chemically ripened by the addition of sodium Iodate.

Tissue processing

Normal spleen, liver and intestinal tissues were obtained from Rabbit. Rapid manual tissue processing was applied as follows: immediate fixation in 10% neutral buffered formalin for 48 hours. They were cut up into small representative portions, put into properly labeled tissue cassettes and subjected to the tissue processing. The tissues were dehydrated through the indicated grades and changes of ethanol for the stipulated period as follows: 70% ethanol (for 2 hours), 90% ethanol I(for 2 hours), absolute ethanol II (for I-2 hours), absolute ethanol III (for I-2 hours). The tissues were cleared in two changes of Xylene (Xylene I for 2 hours, Xylene II for 2 hours). Solid paraffin wax was cut into tiny pieces and placed in a stainless steel plate. The wax was heated using a hot-air oven to 4°C above the melting point of paraffin wax and sufficient quantity of the molten wax was poured into the moulds so as to adequately cover the tissue.The tissues were oriented centrally in the block moulds containing the molten paraffin wax using warm forceps to ensure that tissues are cut in the right planes during microtomy. The mould containing paraffin wax was then covered using the base of the tissue cassette so as to allow the blocks to solidify against the back of the cassette. The molten wax was then allowed to cool using an ice bath. When the blocks set, they were removed from the moulds using forceps. Excess wax was removed from the body of the cassette for easy microtomy. The tissue was placed into the moulds containing three changes of paraffin wax for the following indicated period of time; paraffin I (for 2 hours), paraffin II (for 2 hours), paraffin III (for 4 hours). The blocks were trimmed using a rotary microtome to expose the tissue surface and placed in an ice bath before sectioning with the microtome. Sectioning was done at 5 microns and the sections were then floated onto a hot water bath set at 10°C below the melting point of paraffin wax.

Staining procedure

Staining with the plant products as a primary stain the slide was dewaxed in Xylene and taken to water. It was stained with plant extract staining solution for 15 to 20 minutes and washed in tap water .The slide was dehydrated in ascending grades of alcohol (50%, 75%, 95% and Absolute), cleared in Xylene and mounted with DPX.

Results

The basic cytoplasmic elements of the cell's contents, which range in color from henna brown to black, were readily visible. Ammonia, potassium alum, and acetic acid were added to an ethanolic henna solution to increase Lawsonia inermis' affinity to acidic structure nuclei. According to the general staining principle that only basic stains color the acidic structure nucleus, the alkalinized solution showed improved staining of the acidic nucleus, and henna also faintly stained malaria parasites, indicating that the acidic nature of henna plant extracts was probably responsible for their staining to the basic cytoplasm.

Figure (1): Normal liver tissue stained with fresh 100 mg/ml alcoholic Lawsonia inermis as a primary stain (x10)

Figure (2): Normal spleen tissue stained with fresh 100mg/ml ethanolic *Lawsonia inermis* as a primary stain (with ammonia, x10)

Figure (3): Normal intestinal tissue stained with fresh ethanolic henna show fairly stained cytoplasm but no nuclear staining. (x10)

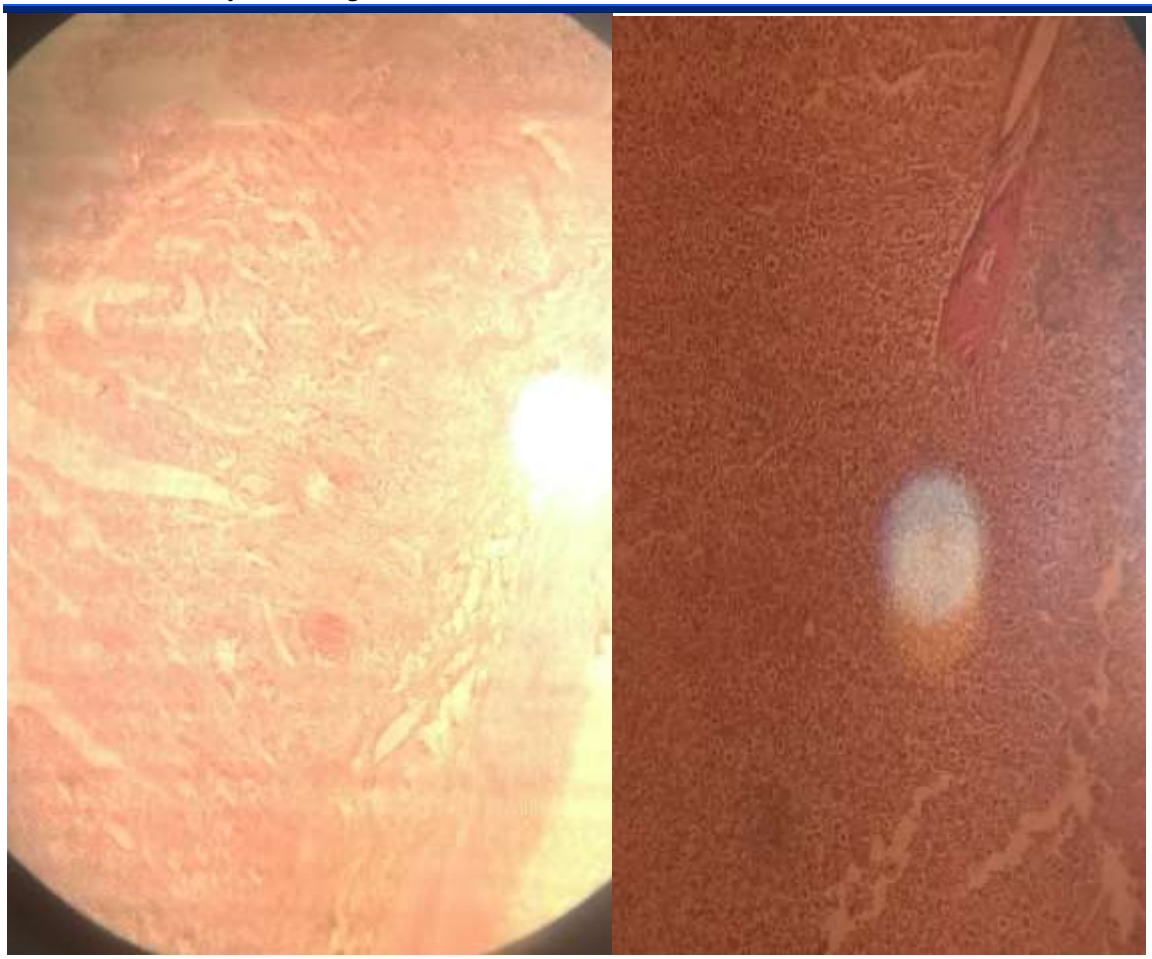

Figure (4): Intestine {the left} and liver {the right} hematoxylin and eosin stained sections (x10)

Figure (5, 6,) Blood smear stained with ethanolic henna. (x100)

Discussion

Despite the fact that henna plants are grown in practically every nation on earth and that it is frequently used as a cosmetic dye for body art that colors the hair, skin, and nails, it has not yet been utilized as a biomedical stain. As a result, this is terrible. The demand

for colors derived from natural sources has increased recently. This is because synthetic colors are toxic and have hazardous chemicals, and because their production and importation are expensive. The current investigation demonstrates that the ability of Lawsonia interims (Henna) to stain histological tissue sections and malaria blood films was unquestionably demonstrated. Our results concur with the Chukwu report on henna use(16) Depending on the staining solution's pH, Lawsonia inermis extract likely served as an acidic or basic stain since, according to Gulrajini ML, when cytoplasm or nuclear structures are of interest, it is necessary to choose an appropriate pH that will facilitate the stain's uptake (17) Hematoxylin and eosin staining of the control section of the colon and liver reveals a distinct distinction with a bluish and pink-red colouring (Figure no 4). Henna extract staining produces a golden brown to light black tint (Figures 1 -2-3). In comparison to Henna extract coloured sections, the morphologic structures of the control intestine and liver sections are more apparent and distinct. All of the blood cells were not colored by henna stain, however the ring or malaria trophozoites were mildly tinted brown Figure (5, 6).

Conclusion

The ability of Lawsonia inermis, a naturally occurring acid dye, to stain both cytoplasmic and nucleus structures on a histological tissue section as well as malaria trophozoites on blood films, was conclusively demonstrated. Henna, however, was well demonstrated to be an efficient counter stain or cytoplasmic stain that can be used in place of eosin in histological sections. Additionally, by adding ammonia, potassium alum, and acetic acid to the ethanolic henna solution, it has a slight potential to stain the nucleus, but it is unable to replace hematoxylin because when they did so in some aspects of the current study, there was no nuclear staining or differentiation, and the intensities and clarity of the normal of the stained areas were reduced.

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