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Histologystaning with an Alternative Natural Dye (Daucus Carota L.)

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Abstract

The study is designed to extract a natural dye from vegetable for staining various biological tissue instead of artificial pigmentation to give a try less expensive alternative dye to synthetic dye ,therefore extract a dye from fresh purple black carrot (Daucus Carota L.) , wherever , taken 250g of chopped roots carrot were putted in 1L distal water , round flask of reflex condenser ,then added 95% of Ethanol as a solvent of anthocyanins dye at 60 ^{0}C for 3 hours , dye was used to stain rabbit lung tissue ,these tissue processed by paraffin embaded technique and section 5 um thickness ,wasstaind with anthocyanin dye showed stainbronchioles epithelium,blood vessels and alveolar cavity with reddish brown color .The level of the stain was not significant different from eosin stain. The result of this study ,purple black carrot dye could be used as an alternative dye for histological stain .

Background

In histology , utilized two types of dye :artificial dye created from chemical reaction and natural dye acquired from nature sources [1] . Histological staining is a process of facilitate tissue examination under microscope which allowed the tissue structure with more details [2]. However ,synthetic dyes are very effective for staining tissue ,but it may pose a risk to human and animal [3]. Thus we thought to find alternative dye from other sources such as plant, roses and vegetable [4].

Asiatic carrot (Daucus Carota L. SSP. SativusvaratrorubenAlef.) also referred as anthocyanin carrot result of their purple black root ,originate in middle Asia and were introduce and cultivated in Europe[5].

Purple black carrots posses the natural pigment ,which is the anthocyanin set up in plants ,especially red, purple and black fruits and vegetable ,the color of these pigment is affected by many factors such as PH ,temperature, light and type of solvent [6].

Objective of the study

extract natural pigment from purple black carrot instead of synthetic dyes that can be used to stain biological tissue of rabbit lung and compare to tissue stained with artificial stain (eosin).

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Materials and methods

Purple black carrots grow in central and northern Iraq in the winter ,the roots of carrot were taken during February, 2020 . As they were washed in a cold water tap and used fresh for extraction .

Extraction

Afresh sample of purple black carrot (Daucus Carota L.) , were taken and chopped into small pieces by a manual metallic food chopper . 250 of chopped roots were putted in a 1L of distal water , in round flask of reflux condenser.

95% ethanol were added to 750 ml of solution as a solvent of anthocyanin pigments,in addition to a magnetic streere .The extraction process were performed at 60^0c for 3 hours .

Lastly the extraction filtered through Wattman No 1 . filter paper ,a purple color clear in extraction ,whereas the residue was left . The extract was stored at the refrigerator at 2-8 ^{0}cuntil use[7]

Histological investigation

Prepared the rabbit lung tissue for histology , fixed the sample of lung tissue by natural buffer formalin , processed for paraffin embedding technique [8] ,section of tissue at 5 um in thickness .

The lung section were stained with anthocyanin dye and eosin stain at 30 min .

result and discussion

all slides were observed under the light microscope with 10X magnification the

stability and quality of carrot dye .

The extracted carrot staining can stain the epitheliumcells of alveolar cavity ,bronchioles epithelium ,and blood vessels with reddish- brown color , structures are extremely obvious , when compared with other acidic stain (eosin) , it’s not differ significantly from eosin in its staining of tissue .

The result indicate that the vegetable dye can be used instead of the synthetic dye without causing any tissue damage.

There are many researches that indicated the anthocyanin have antibiotic, anticancer antiflamentory properties as well as a cardiovascular disease.

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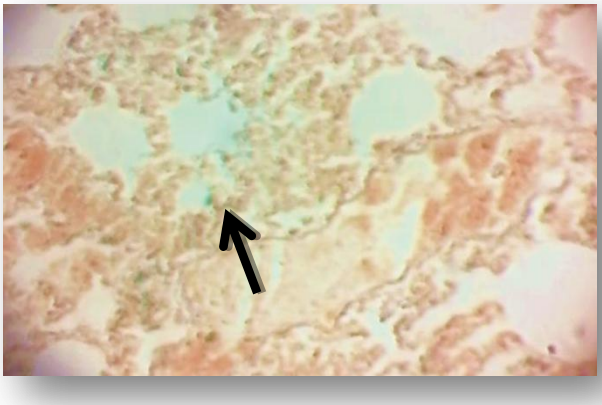
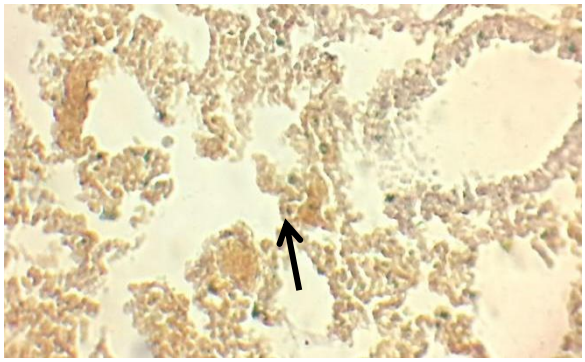
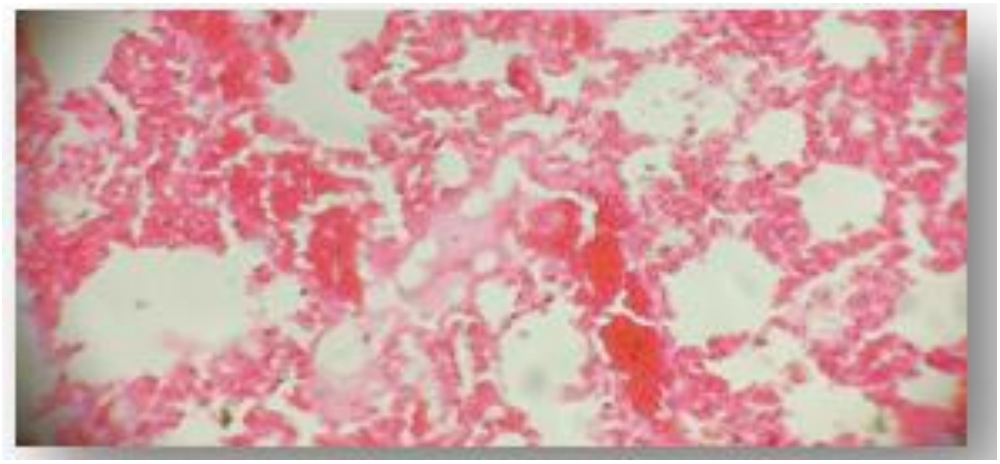


(A)



(B)

Fig (1) A,B section of rabbit lung shows epithelium cells of alveolar cavity , bronchioles.shows redddish brown with carrot stainX10



(c)

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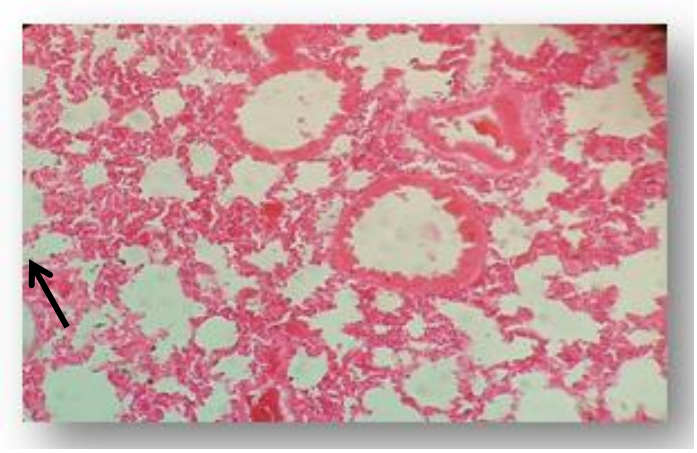

(D)

Fig(2) C,D section of rabbit lung ,shows cavity of alveolar ,bronchioles epitheliumwith pinkish orange color with eosin stain X10.

Conclusion

The purple black dye could stain the blood vessels ,bronchiol and alveolar cavity of the rabbit lung with reddish –brown color ,the optimal staining was form the fresh purple carrot dye extracted at 60 ^{0}c for 3 hours , it's could be used as alternative dye for histological staining the same quality as synthetic dyes for biological tissues pigmentation.

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