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Application Of Juglansregia L. Bark Extractsas An Alternative Dye For Histological Studies

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Abstract

Objective:*Juglansregia*L. (Common Walnut), Juglandaceae, is one of important medicinal plants. It is used to treat skin inflammations, ulcers and venous insufficiency. Its bark is used by Iraqi women to obtain a natural red-brown lip color, but there are no previous studies to use its natural pigment as a histological dye. This study aims to find new natural dye to be as alternative for synthetic dye.

Materials and Methods: Paraffin sections of rat kidney, liver and spleen were prepared according the routine histological work and used in this study.Ethanolic extractof *J. regia* bark was prepared and used as a counterstain after Harris haematoxylin.

Results: After microscopic examination,*J. regia*extract showed strong affinity for the cytoplasm. Cell nuclei were stained by haematoxylin while the cytoplasm was stained by *J. regia* extract. This affinitycan be inferred that the plant extract has an acidic nature due to its polyphenoliccompounds.

Conclusion: *J. regia* extract is a good natural alternative for eosin

Keywords: *Juglansregia*, Juglandaceae, counterstain, haematoxylin, eosin

1. Introduction

*Juglansregia*L. (Walnut), a species from Juglandaceae, is a deciduous tree naturally grows in a wide area of the Carpathian Mountains, Eastern Europe, Turkey and Iraq.Itis long-lived normally 100-200 years,but some specimens may reach 1000 years old. Its bark is grey and deep vertical fissures run along the plant(Topdhan and Lalitkumar, 1994; Ming-Hui*et al*., 2014; Yunus*et al*., 2014).

*J.regia*has an economically important plant because of its high valued timber and edible nutsThis plant is also used in Chinese and European folk medicine to treat skin inflammations, ulcers and venous insufficiency(Gîrzu*et al*., 1998; Chenian*yet al*., 2013;Paola*et al*., 2017).

In histological procedure, stains were used to demonstrate important features of the tissue and to enhance the tissue contrast. Based on their sources, there are two types of stains natural and synthetic stains(Avwioro et al., 2007). Haematoxylin obtained from *Haematoxyloncamechianum*tree is an example of natural dyes, whereas eosin is an example of synthetic stains(Baker and Silverton, 1976; Bhuyan andSaikia, 2005; Hani*et al*., 2016).

Synthetic dyes can be carcinogenic or produce allergy-likesymptoms. Though synthetic dyes are very proficient but theirapplications are restricted due to their harmfuleffect to human and animal health. The use of eco-friendly and biodegradable materials has a worldwide concern(Chengaiahet al., 2010; Hikmat et al., 2011).

Natural dyes obtained from plants are lessexpensive, is being used as a substitute to synthetic dyes especially for developing countries which can no longer afford the increasing cost of synthetic dyes(Avwioro et al., 2007;Sewekow, 1988).

*J. regia*bark is used by Iraqi women to obtain a naturalred-brown lip color, but there are no previous studies to estimate the use of its pigmented extract as a potential histological dye. Based on the aforementioned, this study aims to investigate the use of ethanolic extract of *J. regia*bark as a natural histological dye.

2. Materials and methods

2.1. Plant materials

Dried *J. regia*bark was collected from Iraqi markets in Al- Nassirah city and authenticated as *J. regia*bark by prof. SaharA.A. Al- Saadi, College of Science, University of Basrah, Basrah, Iraq. *J. regia*bark was ground to fine powder for further extraction.

2.2. Plant extraction

Ethanolic extract were prepared according to modified method of Harborne [14]. Crushed bark (20g) was suspended in 200 ml of 95% ethanol (Gainland Chemical Company, UK) and extracted for 4 hours

in the soxhlet apparatus (Indiamart, India). Resulted extract was filtered through Whatman filter paper. The extract was stored in a dark cold place until its use(Harborne, 1984).

2.3. Preparation of sections

Rat liver, kidney and spleen fixed in 10% formalin were obtained from the laboratory of histology and physiology, Biology department, College of Science,University ofThi-Qar. Rat were used as the source of organs obtained from the animal house, Collegeof Science, Thi-Qar University, and were approved by thelocal animal care ethics committee. These sections were dehydrated through 30, 50, 70, 80, 90 and two changes of absolute ethanol for 1 hour each. Clearing was achieved through chloroform (Sigma-Aldrich, USA) for 12 hours. Infiltrating through two changes of paraffin wax at 60℃, then tissue were embedded in paraffin wax. Sections were cut at 5 µm with rotary microtome (Leica RM 2125, Germany) mounted to slides and dried overnight at room temperature(Luna, 1968).

2.4. Staining procedure

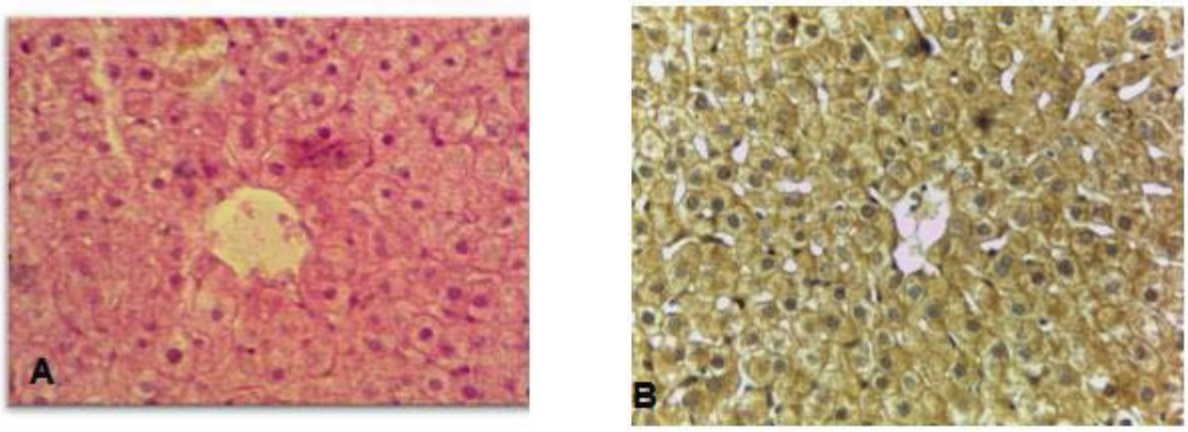
Sections were dewaxed in xylene(Sigma-Aldrich, USA) for 15 min. Then sections were divided into two groups. The first one was served as control group, which stained according to the standard method of haematoxylin-eosin. Sections of the second set were stained according to the following procedure After dewaxing, Sections were hydrated through descending grades of absolute, 90%, 80%, 70%, 50% and 30% ethanol, then Sections were stained in Harris hematoxylin (Syrbio, Syria) for 10 min, washed in running tap water for 15 min, dehydrated in ascending grades ethanol 30%, 50%, 70% and 80%, after that Sections were counterstained with 95% ethanolic extract of *J. regia* for 1 hour, dehydrated in absolute ethanol, cleared in xylene, and mounted in Canada balsam(Sigma-Aldrich, USA), finally Prepared slides were allowed to dry at room temperature.

2.5. Microscopic examination

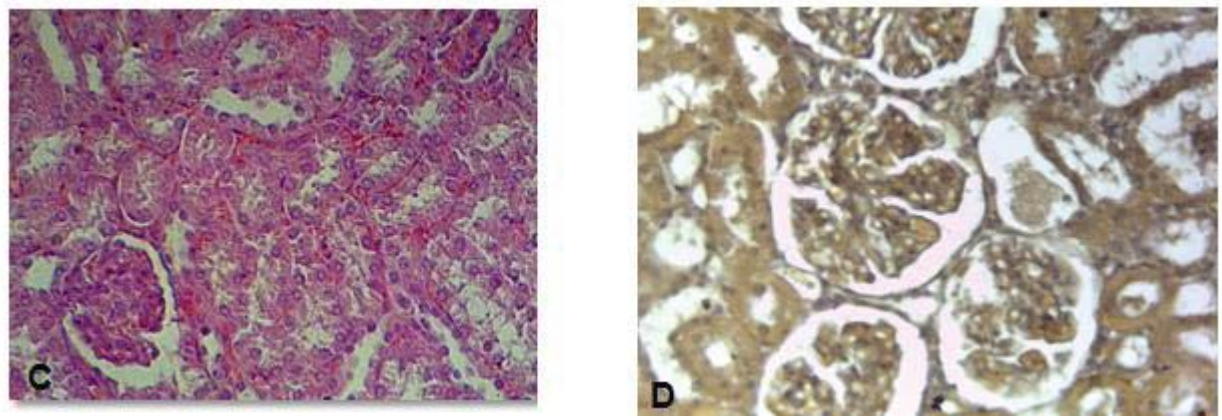
Sections were observed using binocular microscope (MeijiTechno.MT 4200L, Japan) at 10X and 40X magnification.

3. Results and discussion

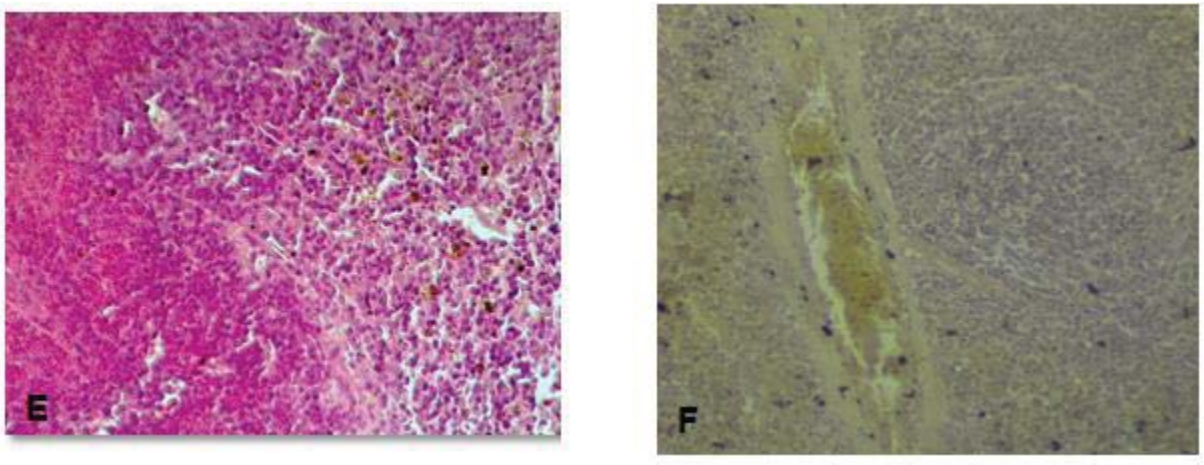
The cytoplasmic content of the liver, kidney and spleen tissues were stained by ethanolic extract of *J. regia*, when it used as a counterstain for haematoxylin. Nuclei of the tissue were stained by haematoxylin dye. The staining reaction of *J. regia* extract was similar to that of eosin except its different color image (A-F).



A. Liver control section stained with haematoxylin-eosin 40X. B. Liver section stained with haematoxylin-*J. regia* extract.40X.Notice the general staining of tissue structures in a manner.



C. control section of kidney (haematoxylin- eosin) 40X. D. kidney section stained with haemoxylin-*J. regia* extract. 40X. Notice the general staining of tissue structures in a manner.



E. control section of spleen (haematoxylin- eosin) 40X. F. spleen section stained with haemoxylin- *J. regia* extract. 40X.Notice the general staining of tissue structures in a manner.

in *J. regia* bark(Mohammad and Loghman, 2013; Mohd*et al*., 2017).

The ability of the dye to stain specific histological structure isdeterminedby certain factors, one of which is the acidity of thestain.Acidic structures would be stained by basicdyes, whilebasic structures would be stained by acidic dyes.The strong affinity of *J. regia* for the cytoplasm can beinferred that the plant extract has an acidic nature. *J. regia* contains flavonoids, which are typicallypolyphenolic compounds and polyphenolic compounds also(Avwioro*et al*., 2007; Faramarz*et al*., 2013).

Phenols are acidic becauseof their ability to release hydrogen from their hydroxyl group(Sachin et al., 2014).So, this gives the extract solution the ability to stain the basic cellular structures.

Conclusions

J. regia extract is a good natural counterstain for haematoxylin. This affinity can be referred to the acidic nature of *J. regia*extract, because of its highly content of polyphenolic compounds.

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