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Alum Hematoxylin and Eosin Stain, Comparison between Bluing in Running Tap water and Ammonia Solution

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Abstract:

Background: Hematoxylin together with eosin (H&E) stain has been the most universal and traditional staining method. The accurate nuclear reaction is essential for tissue evaluation. **Objective:** is to compare between bluing agent of hematoxylin in running tab water at different pH values (6.1, 6.7, and 7.7) against ammonia solution as the standard pH [8.9]. **Materials and methods:** The study included 72 formalin fixed different tissues stained with Mayer's hematoxylin and treated with tap water at different pH values.

Results: the study showed that the nuclear reaction that yielded excellent results was 70%. When compared the nuclear results, with respect to the pH value, there was no difference between the standard and pH 6.1, yet the pH 6.7 and red sea water of pH7.7 gave a minor reaction quality. No significant difference was observed between the quality of nuclear and cytoplasmic reaction; (68%) of cytoplasm gave excellent results. **Conclusion:** As a conclusion all sections, to some extent, gave acceptable results of hematoxylin bluing reaction. The variation in drinking water has no significant variable result of nuclear hematoxylin reaction.

Keywords: Hematoxylin, Nuclear reaction, bluing

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Introduction:

Hematoxylin together with eosin is a widely used histological stain. Its popularity is based on simplicity and ability to demonstrate clearly an enormous number of different tissue structures [1,4].

The staining method involves application of hemalum, which is a complex formed from aluminium ions and oxidized Hematoxylin. This colors nuclei of cells blue [6].

Hematoxylin component stains the cell nuclei blue/black with good intra nuclear details; eosin stains the cytoplasm by pink color [1,4].

Hematoxylin and eosin is used to identify inflammation, Chronic inflammatory, vasculitis or other infectious and inflammatory conditions [10].

Eosin is the most common dye for staining the cytoplasm in histology. It is an acidic dye that binds to basic components of the cell, mainly proteins located in the cytoplasm.

Alum Hematoxylin is chemically ripened with sodium iodate. It can be used as an aggressive stain like any alum hematoxylin, however it is also useful as a progressive stain, particularly in situations where a nuclear counter stain is needed to emphasize a cytoplasmic component which has been demonstrated by special stain and where the acid alcohol differentiation might destroy or de-color the stained cytoplasmic component [3].

During staining alum hematoxylin are usually passed on to a neutral or alkaline solution to neutralize the acid and form an insoluble blue aluminum hematin complex; this procedure is known as bluing.

The bluing solution causes the mordant dye-lake to reform in the tissue and become more permanent.

When tap water is not sufficiently alkaline or is even

acidic, a few drops of strong alkaline solution are used [5].

The biggest objection to Mayer's hematoxylin as used in the past has been that stained slides often fade after 1 to 3 years. This problem was eliminated,

however, when the slides were washed, after the Hematoxylin application, with running water for a minimum of 20 minutes [7].

Bluing solution, practically-wise, includes diluted ammonia water, lithium carbonate and Scott's tap water [8]. These chemical methods are classically used in the bluing step for nuclear hematoxylin reaction. Most of laboratories in recent years use running tap water as bluing agent instead of the chemical solution, yet the source of water may vary according to the salt ingredients; therefore this study aims to compare between the results of chemical and tap water with different pH values.

Material and Methods:

The study was conducted in Khartoum State; as a descriptive study. Seventy two (72) tissue sections were prepared from human organs (Liver, intestine and kidney) fixed in 10% formalin. Mayer's hematoxylin applied on all sections and subsequently treated with the solutions of different pH values, as bluing agents and then stained with Eosin as counter stain and then mounted with DPX mounting media. Microscopic evaluation for all slides performed.

Data analysis Statistical Package for Social Science SPSS computer software was used for data analysis. (Significance level was $P < 0.05$).

Results: The total specimens were 72 sections, equally distributed between liver, kidney and intestine of human organs. The sections treated with different pH values during bluing step (pH 6.1, pH 6.7, pH7.7 and

0.05% ammonia standard solution of value pH 8.9).

Nuclear reaction of all sections in different pH: 51 (70%) were excellent, 15(20%) were Good, 4 (7%) were fair and 2 (3%) gave poor results (Table 1). About description of nuclear reaction according to the pH, the standard and pH 6.1 gave excellent 16 (89%) and 2 (11%) good. The pH value 6.7 distributed as 9 excellent, 5 good, 3 fair and 1 poor. pH 7.7 with a result of 10 excellent, 6 good, 1 fair and 1 poor show (table 2).

Cytoplasmic reaction of all sections in different pH: 49 (68%) were excellent, 7 (10%) were fair, 6 (8%) were poor and 10 (14%) yielded good results (Table 3). The distribution of the 51, classified as excellent, were the nuclear reactions according to tissue type, at variable pH values. Those included, in descending order, the kidney 24 (100%), the intestine 17 (70%), and the liver 10 (40%), vide (table4).

The distribution of the 49 excellent cytoplasmic reaction at different pH values, according to tissue type, were (kidney 24 (100%), intestine 17 (70%) and the liver 8 (33%), as shown in (Table 5).

Table (1): Nuclear reaction of all sections in different pH

Nuclear	**Frequency**	**(%)**

Reaction		
Excellent	51	70.0
Good	15	20.0
Fair	4	7.0
Poor	2	3.0
Total	72	100.0

Table (2): The nuclear reaction according to the pH

pH	**Nuclear reaction**				
	Excellent	**Good**	**Fair**	**Poor**	**Total**
Standard	16	2	0	0	18
6.1	16	2	0	0	18
6.7	9	5	3	1	18
7.7	10	6	1	1	18
Total	51 (70%)	15(20%)	4(7%)	2(3%)	72(100%)

P-value *0.000*

Table (3): Cytoplasmic reaction of all sections in different pH

Cytoplasmic Reaction	Frequency	(%)
Excellent	49	68.0
Good	10	14.0
Fair	7	10.0
Poor	6	8.0
Total	72	100.0

P-value *0.001*

Table (4): Distribution of 51 excellent nuclear reaction according to tissue type at different pH vaues.

Nuclear Reaction	Type of tissue			Total
	Kidney	Intestine	Liver	
Excellent	24 (100.0%)	17 (70.8%)	10 (41.7%)	51
Good	0 (0.0%)	5 (20.8%)	10 (41.7%)	15
Fair	0 (0.0%)	0 (0.0%)	4 (16.7%)	4
Poor	0 (0.0%)	2 (8.3%)	0 (0.0%)	2
Total	24 (100.0%)	24 (100.0%)	24 (100.0%)	72

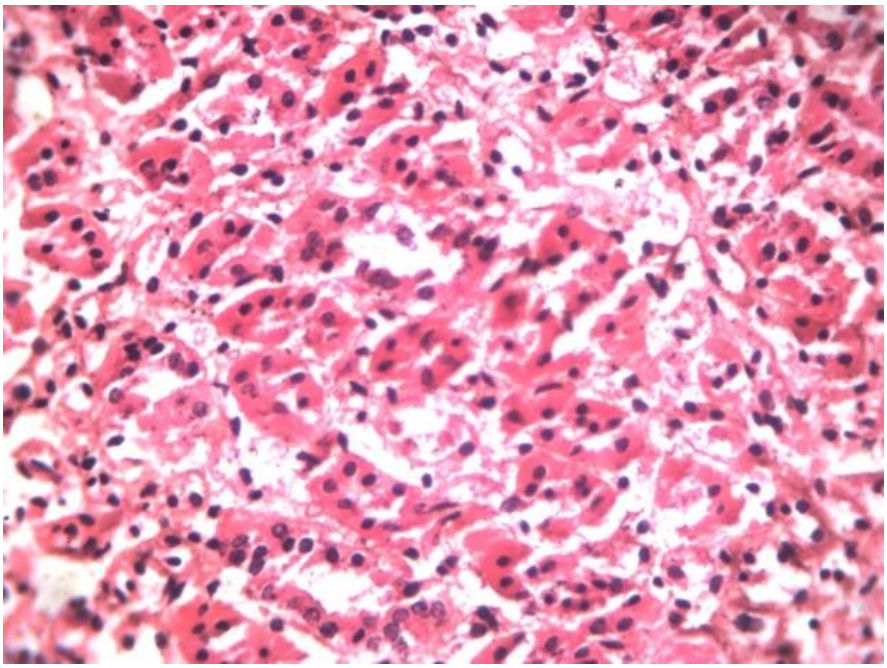
P-value 0.000

Table (5): Distribution of 49 Excellent cytoplasmic reaction according to tissue type in different pH

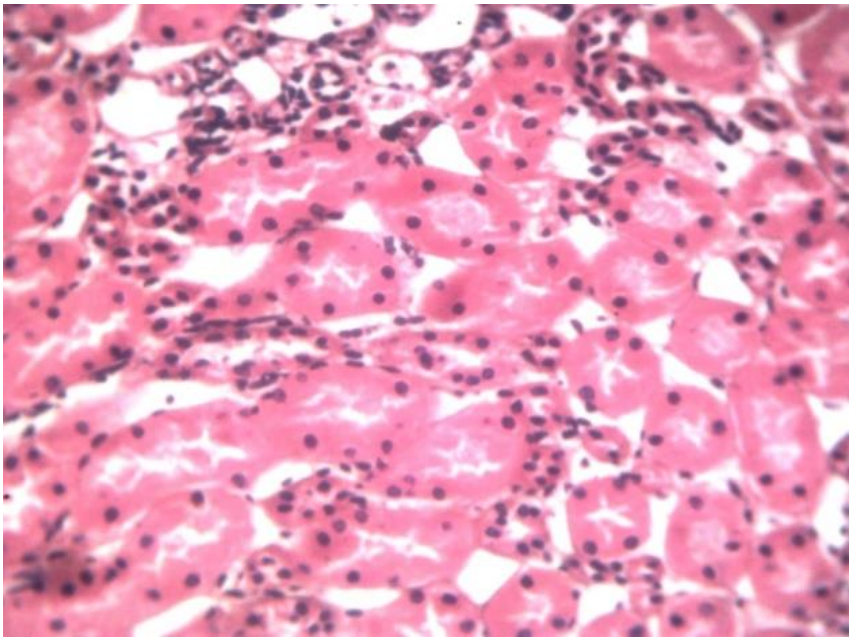
Cytoplasmi	Tissue types	Tota

c Reaction	kidney	Intestine	Liver	**l**
Excellent	24 (100.0%)	17 (70.8%)	8 (33.3%)	49
Good	0 (0.0%)	5 (20.8%)	5 (20.8%)	10
Fair	0 (0.0%)	0 (0.0%)	7 (29.2%)	7
Poor	0 (0.0%)	2 (8.3%)	4 (16.7%)	6
Total	24 (100.0%)	24 (100.0%)	24 (100.0%)	72

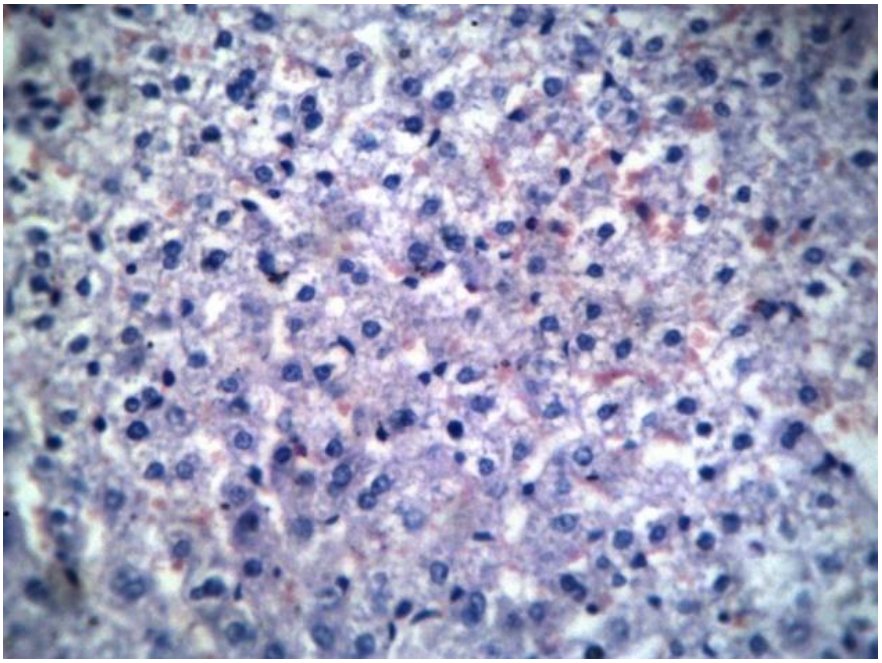
P-value 0.001



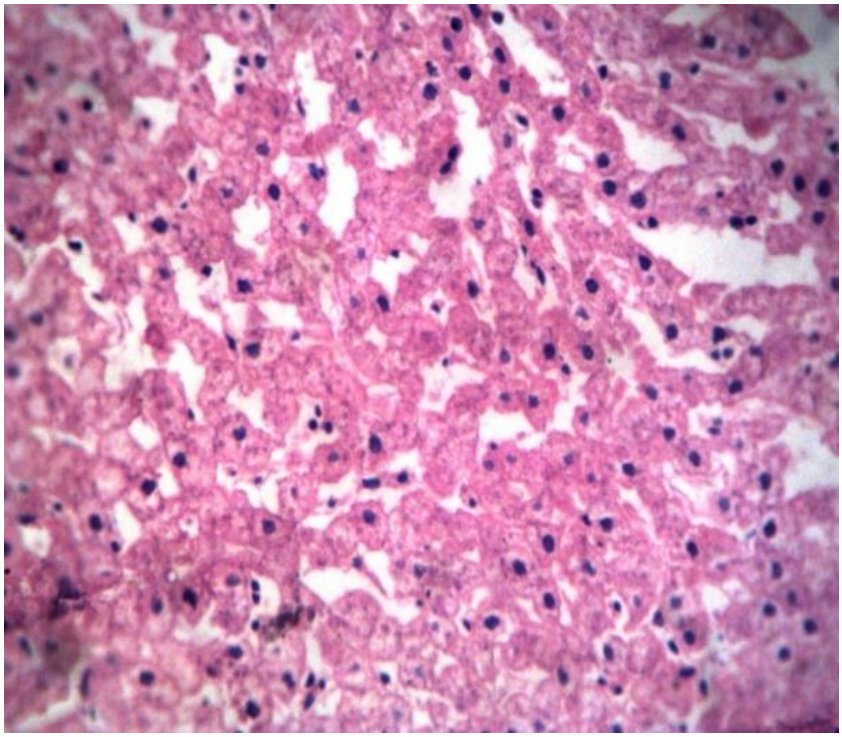
Microphotograph (1): Kidney in Ammonia Solution with Good nuclear and cytoplasm reaction, H&E x40



Microphotograph (2): Kidney in pH 7.7 with fair nuclear and cytoplasm reaction, H&E x40



Microphotograph (3): Liver in pH 6.7 with Good nuclear reaction and poor cytoplasm reaction, H&E x40



Microphotograph (4): Liver in Ammonia Solution with an excellent nuclear reaction, H&E x40

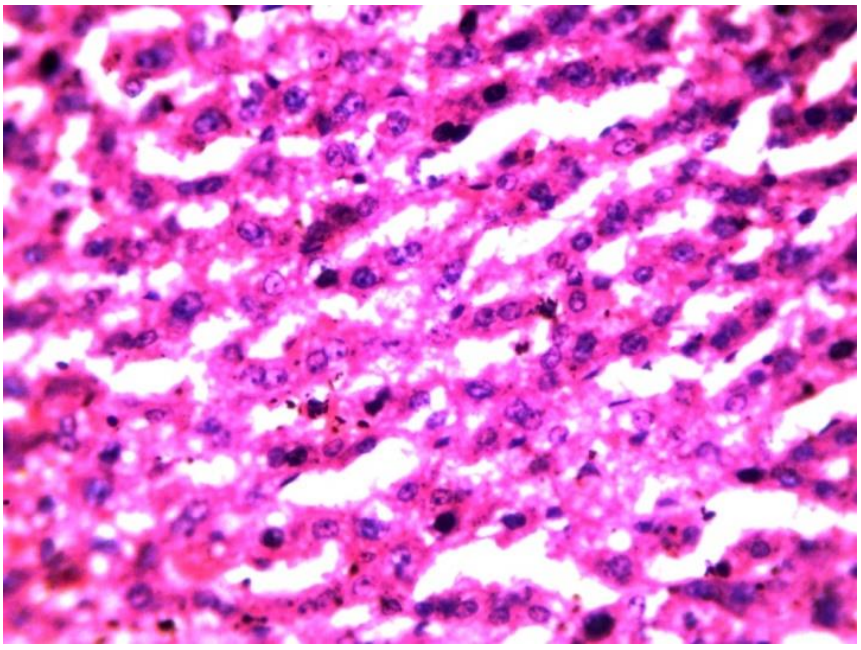


Figure (5): Liver in pH7.7 with poor nuclear and cytoplasmic reaction, H&E x40

Discussion:

There are numerous formulations of bluing reagents available. The active ingredient in any bluing reagent is the alkaline material-either ammonia or alkaline salts such as lithium or magnesium carbonate. The choice of whether to use tap water as a bluing reagent will depend upon the quality of the local tap water [8].

This study was conducted in Khartoum state-Omdurman Islamic University during the period from January to May 2018, to evaluate water bluing of hematoxylin nuclear reaction in different pH values.

The study included seventy two[72] formalin paraffin sections from different tissue types [liver, kidney, and intestine] .the study used Mayer's hematoxylin with eosin stain. The sections subsequently after hematoxylin staining were treated with variable media solutions of different pH values, as bluing step. Those comprised [diluted ammonia solution as standard of pH value 8.9, pH6.1, pH6.7,and red sea water with pH7.7]

All the sections were evaluated microscopically, the result showed that the nuclear reaction with excellent were 70% at the level of *p-value* 0.001. When compared the nuclear results according in terms of the medium, only at the pH 6.7 and red sea water [pH7.7] gave less quality reaction. Although the pH 6.1 was considered acidic, yet it yielded good quality reaction almost similar to the standard. This might be due to the other factors like fixation and processing, because the sections were prefixed and processed. Regarding the cytoplasmic reaction no differences were observed between nuclear and cytoplasmic reaction. The quality of cytoplasmic scored (68%) , and was classified as an excellent result.

As far as the reaction according to tissue type, the study showed that kidney tissue scored a perfect result followed by the intestine and then the liver. This might be attributed to the variations in tissue processing.

As conclusion all sections to some extent gave acceptable results of hematoxylin bluing reaction. The variation in drinking water has no significant variation on the result of nuclear Hematoxylin staining reaction.

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