http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (5): 768-772, 2007
ISSN 1028-8880
© 2007 Asian Network for Scientific Information

Effects Modification of Iron Hematoxylin on Neuron Staining

[1]A.M. Gharravi, [1]M.J. Golalipour, [2]R. Ghorbani and [2]M. Khazaei
[1]Department of Anatomy, Gorgan University of Medical Sciences Gorgan, Iran
[2]Reproductive Research Center, Kerman Shah University of Medical Sciences, Kerman Shah, Iran

Abstract: Iron weigert staining methods is used as nuclear staining. In present study we introduce a modification iron weigert hematoxylin for staining neuron without astrocytes. Whole brain of adult wistar rats (12-13 week old) were removed, immersed in formaldehyde fixative and embedded in paraffin. Sections, 5-7 μm (from brain cortex, hippocampus, cerebellum) divided to three groups: one for staining by Hematoxyllin and eosin, second for staining by cresyl fast violet (that specially performed for Nisl substances in neuron) and last for staining by modification iron hematoxyllin methods, but different in quantity and quality. In new method general and specific architecture of neuron, nucleus and nuclear envelope was clearly visible reactions of neuron were predominant. Astrocyte did not respond to staining methods. Also spines (axon) of purkinje cells clearly visible. Modification iron weigert hematoxylin can be replacement to cost and time consuming chemical staining method for staining neurons

Key words: Neuron, astrocyte, iron hematoxylin

INTRODUCTION

Study of neuropathology has always been marked a certain mystique. This has been partly because of bewildering array of empirical staining techniques (Kiernan, 1999). For nuclear staining; mixtures of mordant dyes with appropriate metal slates are applied to sections of tissue. Hematoxylin can be classified according to mordant: alum, iron, tungsten, molybdenum, lead hematoxylin and without mordant. Also the most common iron hematoxylins are: Weigert, Heidenhain, Loyez and Verhoeff hematoxylin. In Weigert hematoxylin ferric chloride is used as the mordant/oxidant. The main use of Weigert hematoxylin is as a nuclear stain and a greater variety of structural details can be revealed by careful differentiation (Kiernan, 1999; Armed Forces Institute of Pathology, 1960).

Complexity of staining methods of Central Nervous System (CNS) come with complexity of (CNS). For that it is necessary for the researchers of neurosciences to have dyes that can be relied upon to give satisfactory and repeatable results without cost, time consuming and complexity, also it is more necessary for the researchers in growing country to have dyes that can be cost effectiveness in addition to satisfactory results. We investigated modification of iron weigert staining methods, both quantity (replacement of alum with iron) and quality (decreasing staining duration) for staining histoarchitecture of CNS (neurons) to introduce time, cost effectiveness and simplify method that can give satisfactory and repeatable results.

MATERIALS AND METHODS

Preparing tissues samples: For this work whole brain of Wistar-derived strain Adult male rats (12-13 week old and 200-250 g weight) collected. Routine processing for brain performed containing fixation in formaldehyde 10%, dehydrate in ascending ethanol, clearing by xylen, embedding in wax (paraffin) and cutting into 5-7 μm slides (7). Sections (from brain cortex, hippocampus, cerebellum) divided to three groups: one for staining by Hematoxyllin and eosin, second for staining by cresyl fast violet (that specially performed for Nisl substances in neuron) and last for staining by modification iron hematoxyllin methods. In cresyl fast violet method nissl substance, neurons and cell nuclei appeared purple dark blue.

Preparing staining chemicals: For our study we performed material and methods of Rozas iron hematoxyllin (Weigert 1904; Gray, 1954; Rozas, 1935) but different in some chemicals and procedure. We used aluminum nitrate 2 hydrate (AL $(NO_3)_3$ 9 H_2O as an alternative of ferric ammonium sulphate in routine Rozas method. Briefly this material used: Solution A: hematoxylin 0.6 g dye, aluminum nitrate 2 hydrate 1.5 to 2 g mordant, aluminum chloride 1.2 g mordant, distilled water 74 mL solvent 95% ethanol 6 mL solvent, glycerol 20 mL stabilizer. Solution B: aluminum nitrate 2 hydrates 20 g mordant, distilled water 100 mL solvent. For solution A, we dissolved the hematoxylin in the ethanol dissolved the ferric ammonium sulphate and the aluminum chloride in the water. Combined and then added the glycerol.

Corresponding Author: A.M. Gharravi, Department of Anatomy, Gorgan, University of Medical Sciences, Gorgan, Iran
Tel: +98-171-4421651 Fax: +98-171-4421657

Procedure: Briefly this procedure performed: Bringing sections to water with xylene and ethanol. Slide in solution A for 10-20 min. This step is critical for this methods against Rozas iron hematoxyllin method we performed only 10-20 min for12-24 h in this step. Slide in solution B until differentiated. Washing well in running tap water to blue. Rinsing with distilled water. Counter staining, dehydration with ethanol, clear with xylene and mounting.

RESULTS

The results effects of the various staining methods on various areas of brain in adult rats are shown in Fig. 1-5.

Modification of iron weigert stained nucleus of cells black. Also background of sections was cinereous. Timing of staining when compared with Rozas (1935) iron weigert method was ten times lower. Results of neurons and glia staining were different. Positive reaction to neurons and poor reaction to astrocytes appeared by performing this method.

Also results of staining In CA1 of hippocampus with population of pyramidal, molecular, polymorph neurons was different from dentate gyrus with population of astrocyte and neurons. In brain cortex only neurons has seen, while glia have stained poorly. Detailed characteristics contain nucleus, nucleolus, nuclear envelope and plasma membrane clearly appeared of

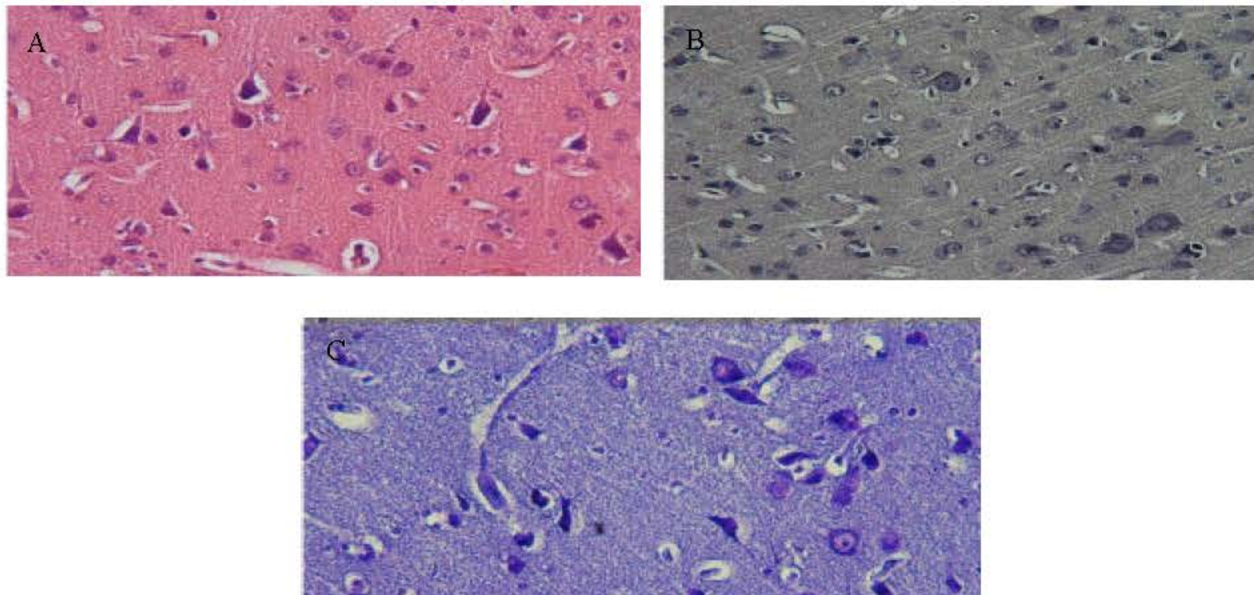

Fig. 1: Results staining cortex of cerebrum by three methods, A) Hematoxylin and eosin, B) Modification iron hematoxylin, C) Cresyl fast violet

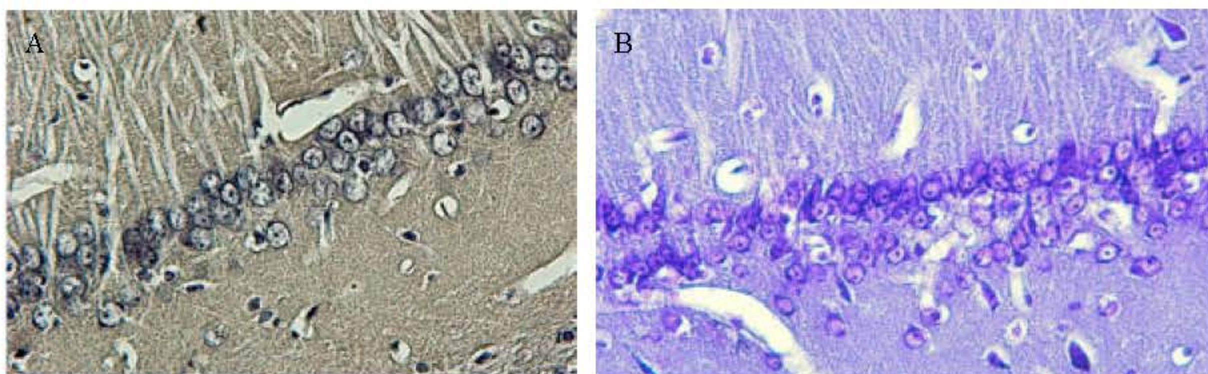

Fig. 2: Results staining C1 of hippocampus by two methods, A) Modification iron hematoxylin, B) Cresyl fast violet

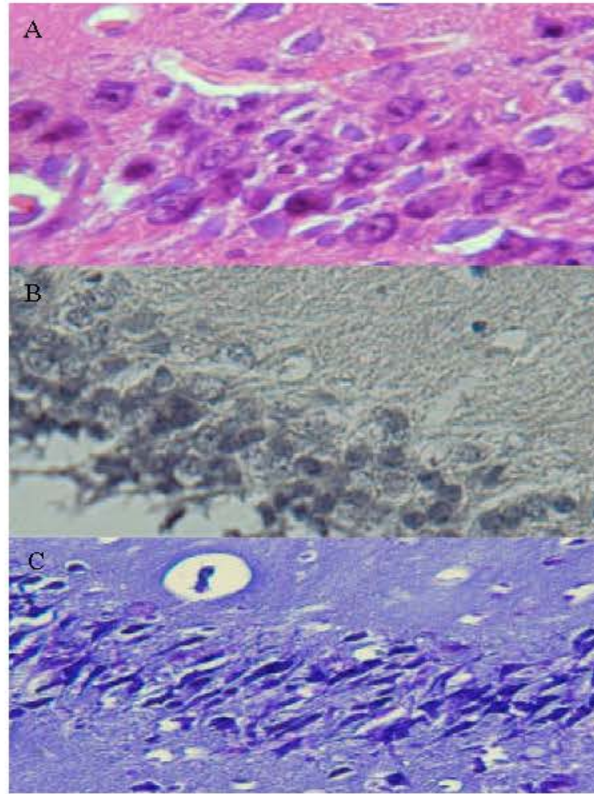

Fig. 3: Result staining dentate gyrus by three method, A) Hematoxylin and eosin, B) Modification iron hematoxylin, and C) Cresyl fast violet

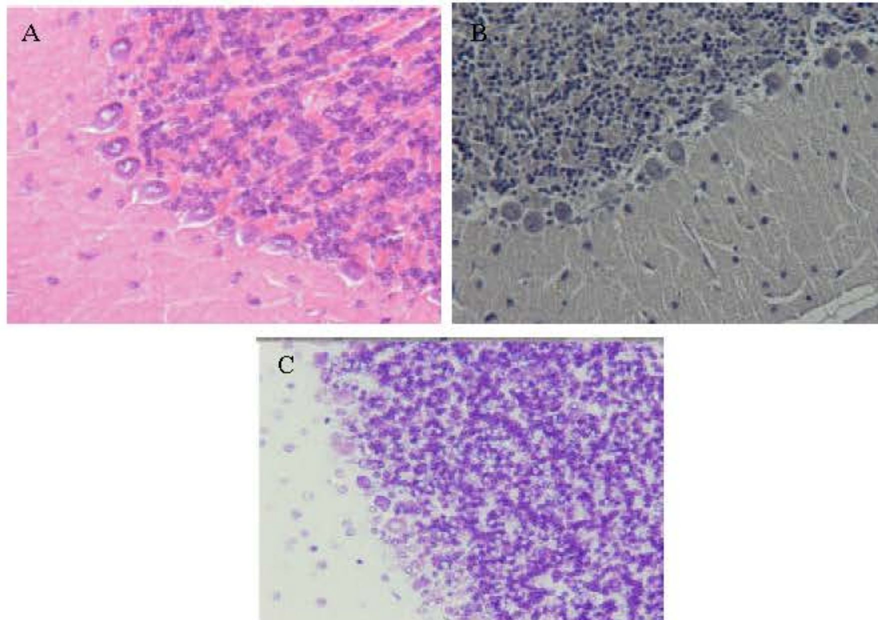

Fig. 4: Result staining cortex of cerebellum by three method, A) Hematoxylin and eosin, B) Modification iron hematoxylin and C) Cresyl fast violet

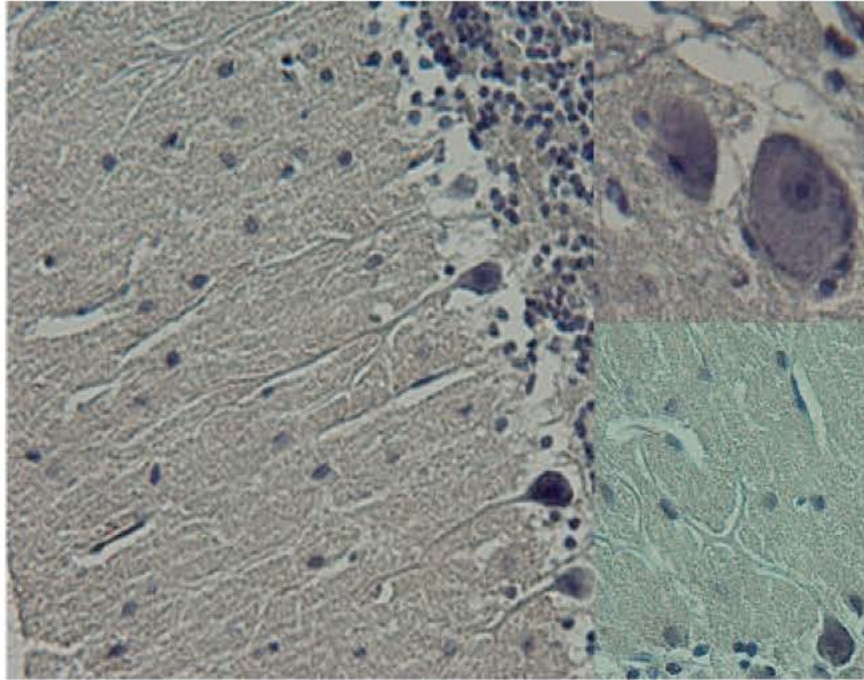

Fig. 5: Results staining cortex of cerebellum by Modification iron hematoxylin A) Spines of purkinje cells. B) Neuron with clearly visible nucleus, nucleolus, nuclear envelop and C) Plasma membrane and glia surrounding it

purkinje and granular cells of cerebellum cortex. Also spines (axon) of purkinje cells clearly visible (Fig. 5).

But distinguish golgi cells in granular layer could not detected by this method.

DISCUSSION

Objective of the study were modification of iron weigert staining methods, both quantity (replacement of alum with iron) and quality (decreasing staining duration) for staining histoarchitecture of CNS (neurons) to introduce time, cost effectiveness and simplify method that can give satisfactory and repeatable results. Results of study support our objective, because this method is uncomplicated and time and cost value and can be used as an alternative method especially for neuron staining in histology and pathology departments.

Fortunately in study of neuropathology empirical techniques are being replaced by reliable immunohistochemical methods. But for growing countries uses of these methods are cost consuming. For nuclear staining, mixtures of mordant dyes with appropriate metal slates are applied to sections of tissue. Hematoxylin solutions can be classified according to which mordant is used: alum, iron, tungsten, molybdenum, lead hematoxylin and without mordant (Kiernan, 1999). Also the most common iron hematoxylins are: Weigert, Heidenhain, Loyez and Verhoeff hematoxylin. In Weigert hematoxylin ferric chloride is used as the mordant/oxidant. The main use of Weigert hematoxylin is as a nuclear stain and a greater variety of structural details can be revealed by careful differentiation. In present study we replaced iron mordant by alum mordant [aluminum nitrate 2 hydrate (AL $(NO_3)_3 9$ H_2O] in weigert staining method. Alum hematoxylin staining may be progressive or regressive. The former is usually preferred (Kiernan, 1999). The binding of dyes to textiles has been studied for many years, but is still not fully understood. Also documents related to modification of iron hematoxylin and replacement of alum mordant by alum mordant is poor. For this reason we could not compare our result with any pervious finding, because of different study design that we performed. Little is known of the mechanism of nuclear staining by iron-hematoxylin. Also little is known about the mechanism of modification iron weigert. Horobin (1998) suggests that dye-metal complexes bound to chromatin by both ionic and non-ionic forces. A number of dyes have been recommended as substitutes for hematoxylin, particularly during the early to mid-1970s when hematoxylin was in short supply. Although some closely mimic the features of hematoxylin, none will serve as a complete replacement (Lillie *et al*., 1973). We recommended time alteration to get better and detailed characteristics of cells because staining time have different results.

Intact determination detailed characteristics contain nucleus, nucleolus, nuclear envelope and plasma membrane of neurons clearly appeared by modification of iron weigert staining methods. It was specifically responded in the nucleus neurons. Neither astrocyte responds to methods. Also cell spines (axons) in particular purkinje cells can be distinguished.

REFERENCES

Gray, P., 1954. The Microtomist's Formulary and Guide. Originally Published By: The Blakiston Co.Republished by: -Robert E. Krieger Publishing Co.

Horobin, R.W., 1988. Understanding Histochemistry: Selection, Evaluation and Design of Biological Staining. Chi Chester: Ellis horwood.

Kiernan, J., 1999. A Histological and Histochemical method, 3rd Edn., Pergamon Press, pp: 31-114.

Lillie, R.D., P. Pizzolato, R.A. Welsh, N.D. Holmquist, P.T. Donaldson and C.A. Berger, 1973. Consideration of substitutes for alum hematoxylin in routine histologic and cytologic diagnostic procedures. Am. J. Clin. Pathol., 60: 817-19.

Rozas, 1935. Zietschrift für wissenschaftliche Mikroskopie und für mikroskopische Technik, Leipzig, 52: 1.

The Armed Forces Institute of Pathology. Manual of Histologic Staining Methods of 3rd Edn., 1960. American Registry of Pathology McGraw Hill Publishers, New York (Progressive Stain).

Weigert, K.E.K., 1904. Verbesserung der Hämatoxylin-van Gieson-Methode. Z. Wiss Mikr, 2L1-5.