

Special Immunohistochemical and Histological Demonstration of Neurotoxicological Effects of Regal Dry Gin on Nuclei Aggregation in the Brain Concerned with Motor Functions

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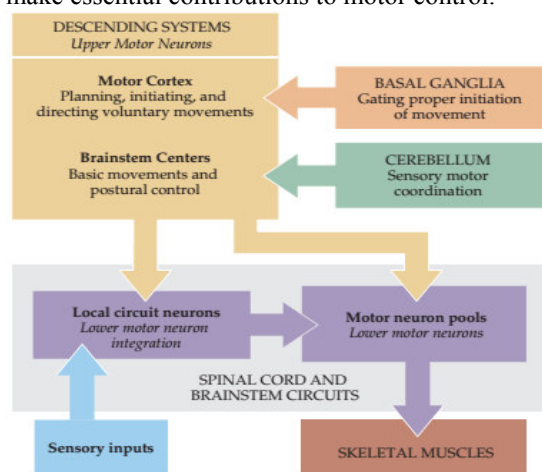
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Abstract

As in most complex systems little disruptions to the brain substance can lead to significant functional disruptions. Properties leading to the susceptibility of nervous tissue include a high surface area of neurons, a high lipid content which retains lipophilic toxins, high blood flow to the brain inducing increased effective toxin exposure, and the persistence of neurons through an individual's lifetime, leading to compounding of damages.^[1] As a neurotoxin, alcohol has been shown to induce nervous system damage and affect the body in a variety of ways. Among the known effects of alcohol exposure are both transient and lasting consequences. Some of the lasting effects include long-term reduced neurogenesis in the hippocampus,^{[2][3]} widespread brain atrophy,^[4] and induced inflammation in the brain.^[5] Since degree of neurodegeneration varies with dosage taken, by some specific immunohistochemical and histological stains we have been able to quantitatively demonstrate the degree of neurodegeneration (nerve cell bodies and fibers degeneration) and extent of and actual apoptosis in acute toxicity of regal dry gin at LD_{50/14} in brain nuclei aggregations concerned with movement in terms of initiation, processing and coordination.

INTRODUCTION

Four distinct but highly interactive motor subsystems—local circuits in the spinal cord and brainstem, descending upper motor neuron pathways that control these circuits, the basal ganglia, and the cerebellum—all make essential contributions to motor control.



Overall organization of neural structures involved in the control of movement. Four systems—local spinal cord and brainstem circuits, descending modulatory pathways, the cerebellum, and the basal ganglia—make essential and distinct contributions to motor control. *Adapted from page 372, Textbook of Neuroscience, Third Edition. Edited by Dale Purves et al.*

The Alpha motor neurons are located in the spinal cord and in the cranial nerve nuclei in the brainstem and directly link the central nervous system and muscles, with each motor neuron and its associated muscle fibers constituting a functional entity called the motor unit. Motor units vary in size, amount of tension produced, speed of contraction, and degree of fatigability. Because of their essential role in all of these circuits, damage to lower motor neurons leads to paralysis of the associated muscle and to other changes, including the loss of reflex activity, the loss of muscle tone, and eventually muscle atrophy.^[4] Alcohol generally affects the brain cortices and various parts of the motor unit including the hippocampal formation and the associated cingulate and dentate gyri.^[3]

MATERIALS:

- Fixatives:
- i. Transcardial perfusion with 30ml of 10% formalin
 - ii. 30ml of saturated picric acid
- Cryo preservatives (in fridge) for 72 hours: i. 70ml of 10% formalin

Orogastric tube and 1ml calibrated insulin syringe.
Beddings, saw dusts and regular water.

METHODOLOGY AND EXPERIMENTAL STUDY DESIGN

Cohort study.

GROUPS

There were 2 groups out of which 10 animals were in the control group and another 10 animals in the treatment group all under the same experimental conditions and temperature.

Protective Measures (Disposables)

Soap; nylon hand gloves with a pair of non-powdered latex gloves worn on it often; hand sanitizers, and hand wipes.

Feeding: Pelletized feeds and clean drinkable water.

Housing: Well ventilated cages with dimensions of 33.0 x 20.5 x 19.0 cm.

Digital weighing scale, Regal dry gin (200ml bottles)

All experimental procedures followed the recommendations provided in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and Published by the National Institute of Health (NIH, 1985).

Treatment Plan

The animals were induced every day from 17th February to 3rd March, 2015 at about 9a.m routinely when they were less active (before sunrise). The water and feeds being changed daily with fresh ones for hygiene purposes.

Gross Anatomical changes

Partial blindness was observed in about 60% of animals in the treatment group.

Results



Fig.1: A - NSE immunohistochemical stain showing a section of the Hippocampal formation in the treatment group $\times 40$ Mg. B - Same hippocampus with NSE immunohistochemical stain in another animal in the same treatment at 100Mg. C Same section through the control group $\times 40$ Mg; Compare. All showing the Dentate and the cingulate gyri.



Fig.2: Treatment group. Hippocampus ×40MG SHOWING CINGLATE AND DENTATE GYRI NEURODEGENERATION

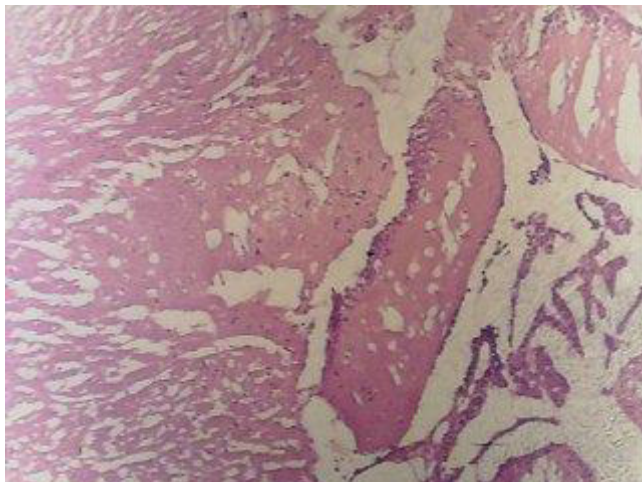


Figure 3: Neurodegeneration adjacent to the lateral geniculate nucleus



Figure 4: Section through the midbrain at the level of the superior colliculus showing vacuolations and neurodegeneration of the cells in red nucleus. H&E ×100Mg



Figure 5: Showing vacuolations around pyramidal cells of the cerebral cortex.

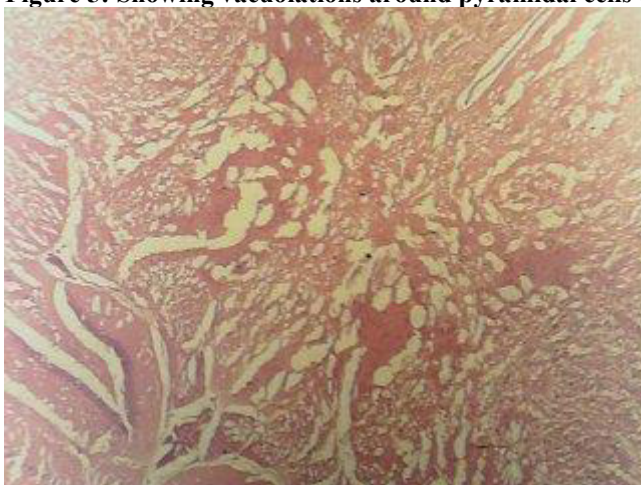


Figure 6: Control group. Slide showing non-vacuolations and non-degeneration in a section of the hippocampal formation using H&E histological stains.



Figure 7: Control group. An immunohistochemical stain section through the cerebellum in the control group showing the activation of glial cells- the astrocytes. $\times 40Mg$

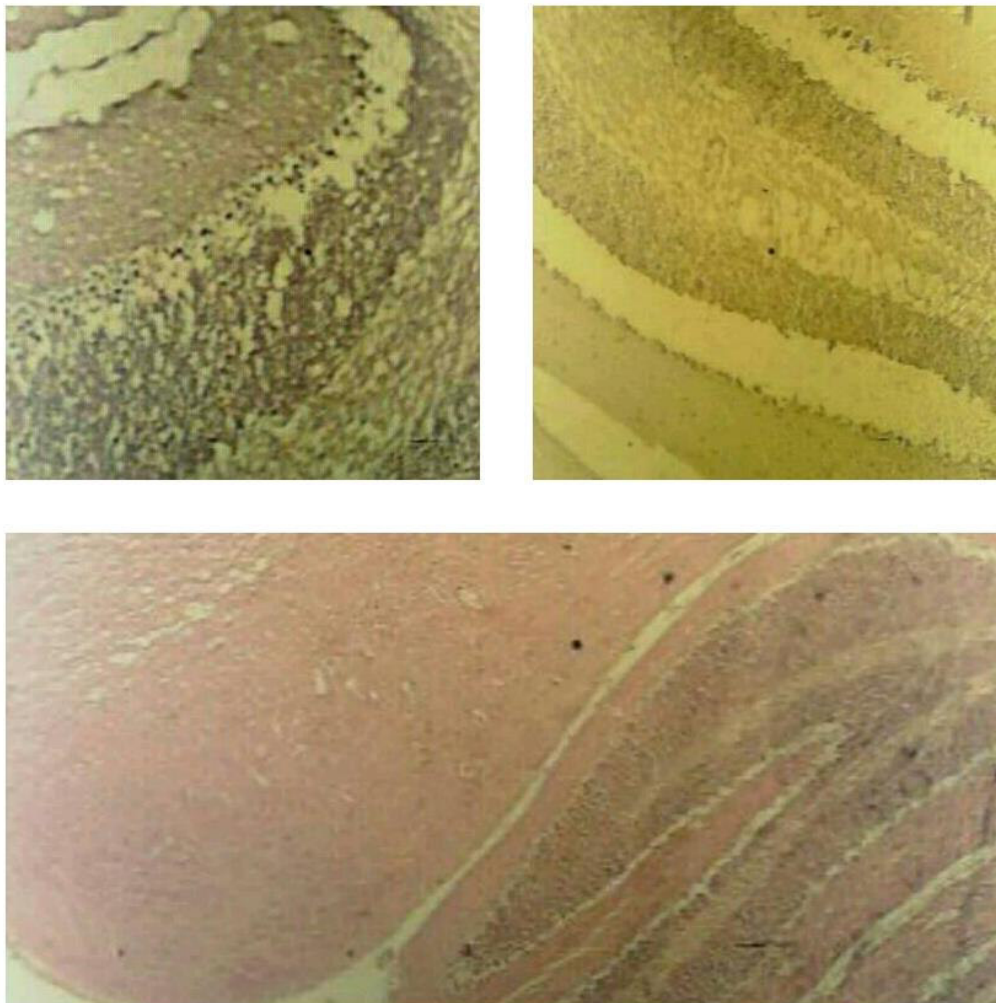


Figure 8: A – Treatment GROUP. NSE immunohistochemical stain focusing the cerebellar cortex. Shows depletion of neurons in the purkinje cell layer Cerebellum. ×100Mg. B – NSE for Treatment group ×40Mg. C – the cerebellum showing no vacuolations and non-neurodegeneration×40Mg.

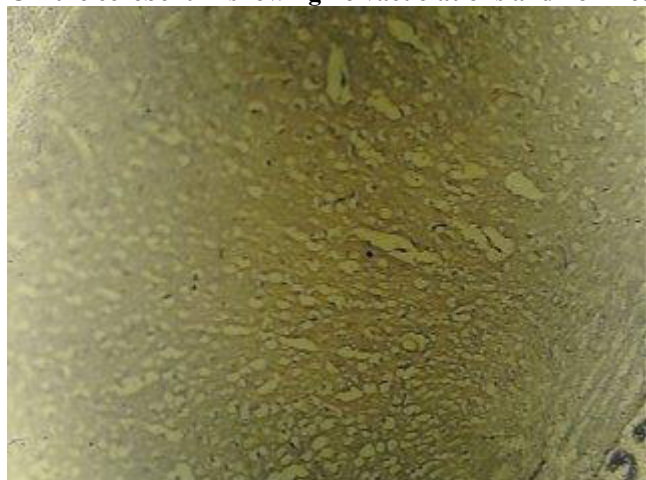


Figure 9: Treatment group. Section through the midbain in a sagittal manner showing activation of astrocytes in this region. × 100Mg



Figure 10: GFAP-Treatment group. Section adjacent the Hippocampus showing activation of astrocytes and their metastasis. ×100Mg

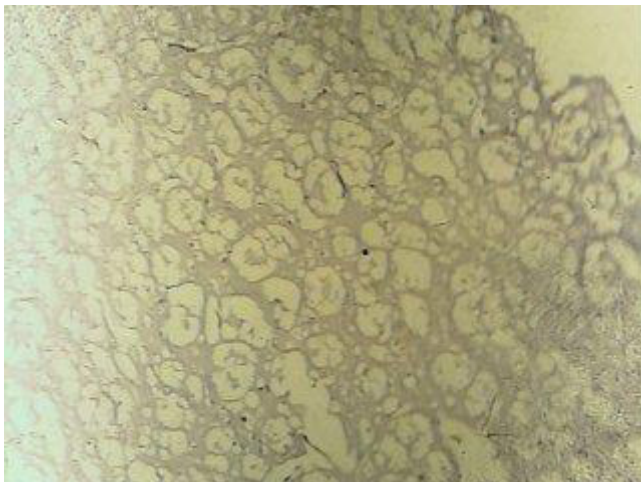


Figure 11: Treatment group; Section through the Red Nucleus showing various glial cells activation with vacuolations around the perikaryon of the neurons. ×100Mg



Figure 12: Treatment group. Section through the Cerebellum showing neurodegeneration around the Purkinje cell layer. ×100Mg

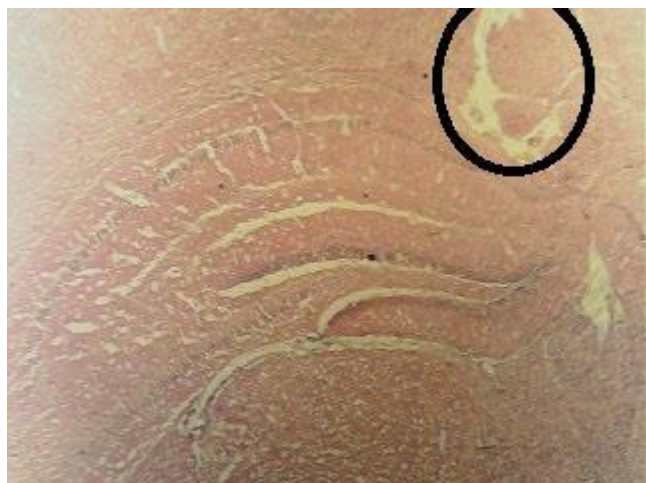


Figure 13: Black circle encapsulating the vacuolations extending to the lateral geniculate body. ×40Mg

CONCLUSION

The pyramidal neurons of the cerebral cortex are also grossly implicated in acute toxicity has seen under the microscope in the animals in the treatment group.

However, of the three layers of the cerebellar cortex, the purkinje cell layer and their projections suffer the most insult in neurotoxicity of alcohol. Few neurodegeneration are also observed in acute neurotoxicity in the molecular layer. This is evident in all the vacuolations in the stroma of the brains of the mice in the treatment group.

Thus, by some specific immunohistochemical and histological stains we have been able to scientifically demonstrate the extents of nerve cell bodies and fibers degeneration and actual apoptosis in acute toxicity of regal dry gin in different places of nuclei aggregation in the substance of the brain.

Finally, chromatolyses were observed in the neural cell bodies at 100 magnification of the animals in the treatment group (Figs 4, 5, 8a and 11, compare with 8c). Also, degeneration was observed to spread to the most ventral part of the lateral geniculate body dorsal to the curvature of the gyri of the hippocampal formation. (Fig.8).

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Further readings

<http://alcalc.oxfordjournals.org/> (Alcohol and Alcoholism)

<http://www.ncbi.nlm.nih.gov/books/NBK53356/>

<http://www.ncbi.nlm.nih.gov/books/NBK53356/> (Advances in the Neuroscience of Addiction, 2nd edition)

<http://en.wikipedia.org/wiki/Neurotoxin>

<http://www.journals.elsevier.com/neurotoxicology/> (Neurotoxicology)