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

Emmanuel O. Adegbite, Dr. J.O. Sanya.
Department of Medicine and Surgery, College of Medicine and Health Sciences, Afe Babalola University

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. 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, widespread brain atrophy, and induced inflammation in the brain. 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 actual apoptosis in acute toxicity of regal dry gin at LD₅₀/₁₄ 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. Alcohol generally affects the brain cortices and various parts of the motor unit including the hippocampal formation and the associated cingulate and dentate gyrus.

MATERIALS:
Fixatives:
1. Transcardial perfusion with 30ml of 10% formalin
2. 30ml of saturated picric acid
Cryo preservatives (in fridge) for 72 hours: 1. 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

![Image 1](image1.jpg)

Fig.1: A - NSE immunohistochemical stain showing a section of the Hippocampal formation in the treatment group ×40Mg. B - Same hippocampus with NSE immunohistochemical stain in another animal in the same treatment at 100Mg. C Same section through the control group ×40Mg; Compare. All showing the Dentate and the cingulate gyri.
Fig. 2: Treatment group. Hippocampus ×40MG SHOWING CINGULATE 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. ×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
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).

AKNOWLEDGEMENT
I want to appreciate first and foremost the Source of all inspirations, the Lord Jesus Christ for overseeing and seeing me through the course of this research. [1 Peter 1:1-3]. I give all glory, honor and adoration to the Lord that maketh ways in the wilderness, the Lord that opens and no man can shut.

I want to appreciate the founder emeritus of my ALMA MATA, Aare Emmanuel Afe Babalola who provided a research environment conducive for development of young and brilliant minds in our highly reputable and classic serene citadel of learning. Also, will I appreciate the incessant and unquantifiable support I receive from the Yeye Aare Modupe Babalola for her motherly care and support at all times.

I want to indeed thank and sincerely appreciate Professor Michael Ajisafe, the vice chancellor of my institution for his full support and love for me always.

Worthy of being noticed is the fatherly love and guidance of a Pastor, a Provost and a Physician in person of our requisite Dr Olurotimi Sanya who stooped low to be of help to a young scientist.

I want to thank my lecturers who introduced me to the nitty-gritty of research principles and concepts, guiding me through most stages, I couldn’t have done this on my own- Mr Phillip Adeniyi, Dr Ogundele, Mr Adekeye Adeshina, Mr Enye Linus Anderson (ELA), Mr Chris Ajonijebu, Mr Toba, Mr Azeez, Mr Adams Omohage I can’t quantify your help.

I also want to appreciate the efforts of my colleagues who are co-workers for this project, Brother David Daniel (for being humbly there when needed always), Fatona Bola, Mr Dare, Olaitan Virtue, Jemima Ovie, Suraj and Dami.

Importantly, I want to thank God for and appreciate Miss Itamunoala Emmanuela Belema who stood beside me in good and bad times all through. May the good Lord bless the union bountifully.

References

Further readings
http://alcalc.oxfordjournals.org/ (Alcohol and Alcoholism)
http://en.wikipedia.org/wiki/Neurotoxin
http://www.journals.elsevier.com/neurotoxicology/ (Neurotoxicology)