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RESEARCH ARTICLE

ELECTROPHYSIOLOGICAL APPROACH AND NEURO BEHAVIOURAL ASSESSMENT OF ACUTE NEUROTOXICITY WITH REGAL DRY GIN

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ABSTRACT

Electrical and behavioural effects of exposure to lethal dose level of regal gin have been examined in mice.Regal gin is ubiquitous and a widely consumed drink among youths. Its major component, alcohol is implicated in the aetiology of several neurological disorders and likewise known to cause various damages to different parts of the brain, chronically and acutely.The treated mice show significant slow response to motor stimuliand prolong response time even after the withdrawal of stimuli. Behaviour of both the mice was examined by ethological analysis of encounters between the gin-treated and control mice of the same sex. It suggests that acute neurotoxocity with regal gin causes significant alteration in motor function and the limbic system at the level of the hippocampal formation, thus producing significant behavioural changes between the two groups. Significantly, similar effects were widely spread among animals in both groups. In this study, the trend of action potential (AP) generated is discussed in different phases. Spike trends and peak patterns were also carefully read and noted.

INTRODUCTION

Regal dry gin is widely taken among youths across all races, ethnic groups and even gender. Its major constituent is known to have addictive properties and causes damage to parts of the brain. Techniques such as electroencephalography (EEG), event-related potentials (ERP), and brainstem evoked response (BSER) all share a common approach to cortical electrophysiology –scalp electrodes are used to detect electrical activity generated bythe brain.

These techniques can provide insights into brain -behavior developmental issues that complement and supplement information obtained through more traditional behavioral measures. This approache compare to other techniques, such as Positron Emission Tomography (PET), Magnetic Resonance Imaging (MRI), and functional Magnetic Resonance Imaging (fMRI).[1]

The raw data was read using Audacity, SigView, PClamp 10.5, Clampfit 10.5 and the AxoScope Scope-Demo Digitizer.

This study examined the effect of regal gin on patterns of neural activity generated by freshly isolated cortical tissues to propose an in vitro electrophysiological approach to studying neural functions. We hope to describe cytotoxic pathways (if possible) associated with regal gin induced neural dysfunction.

Electrophysiology

The study made use of the SpikerBox from Backyard Brains(www.backyardbrains.com). The device was connected to a computer interface (Audacity). Stimulation was at 1.5 mV and the action potential generated was recorded as spikes on the Audacity software. This was done on the motor cortex.

Spectrum analysis

The recorded spikes were processedusing the menu on the audacity software (pc version). The project rate was set at 44100 Hz and the spike regions were trimmed out and zoomed to an interval of 0.005s. The peak patterns were selected at regular intervals and analyzed by spectrum plotting to generate an algorithm in enhanced autocorrelation through which the frequency in db at 0.00 to 500 ms at an interval of 50ms were obtained. The spectrum plot was later depicted in the Hanning window to show peak patterns at the recorded intervals.

[1]Molfese, D.L., Molfese, V.J., & Kelly, S. (2001). The use of brain electrophysiology techniques to study language: A basic guide for the beginning consumer of electrophysiology information. Learning Disabilities Quarterly, 24, 177 -188.

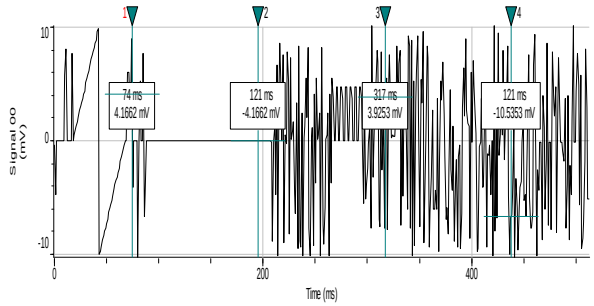

Figure 1 Graph showing variations in a fluctuating electrical quantity appearing in visible wave forms (using a cathode ray tube) in an animal in the Control group.

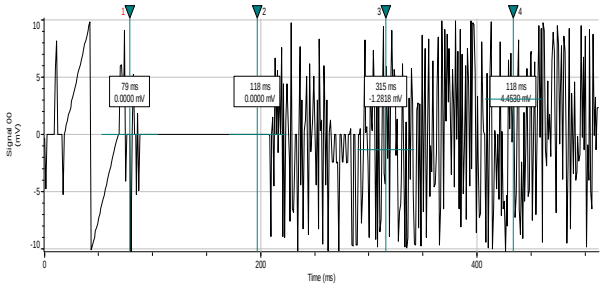

Figure 2 Graph showing variations in a fluctuating electrical quantity appearing in visible wave forms (using a cathode ray tube) in another animal in the Control group.

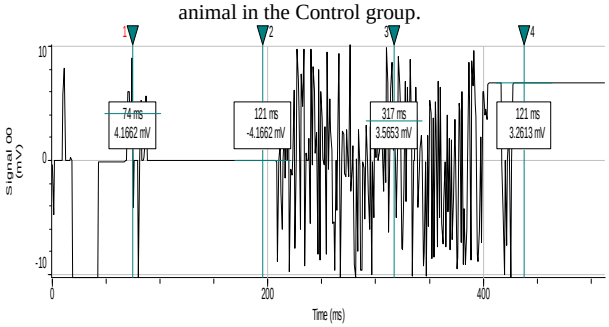

Figure 3 Graph showing variations in a fluctuating electrical quantity appearing in visible wave forms (using a cathode ray tube) in an animal in the Treatment group.

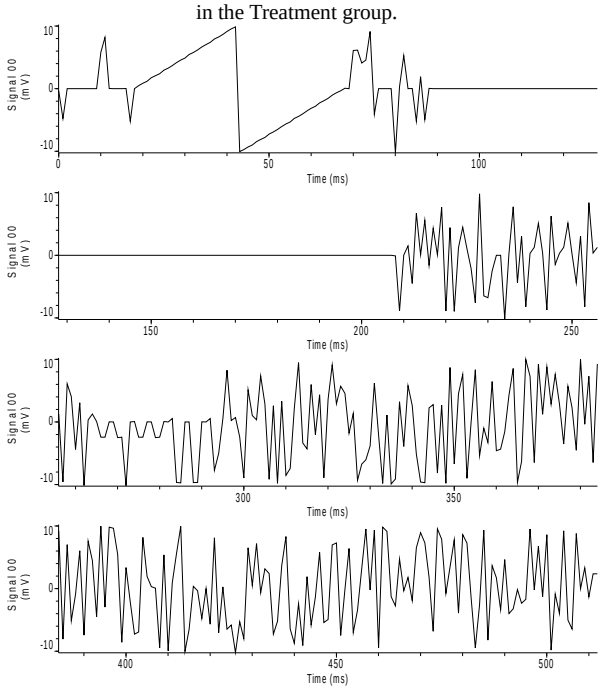

Figure 4A Graph showing variations in a fluctuating electrical quantity appearing temporarily in visible wave forms (using a cathode ray tube) in an animal in the Control group. This shows timed wave pattern with readings at the motor cortex.

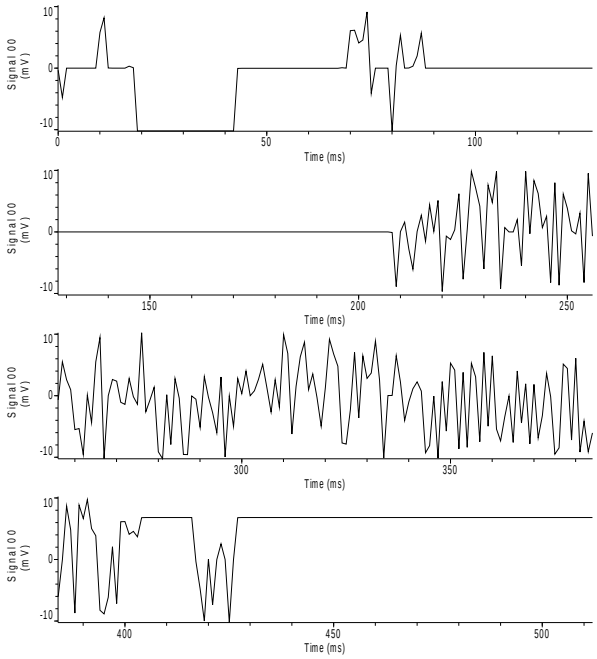

Figure 4B Graph showing variations in a fluctuating electrical quantity appearing temporarily in visible wave forms (using a cathode ray tube) in an animal in the Control group. Compare wave pattern from 400ms in A and B. Compare with wave patterns at 400ms when tapped, this shows slow response in initiation of movement.

Power Spectra From Clampfit

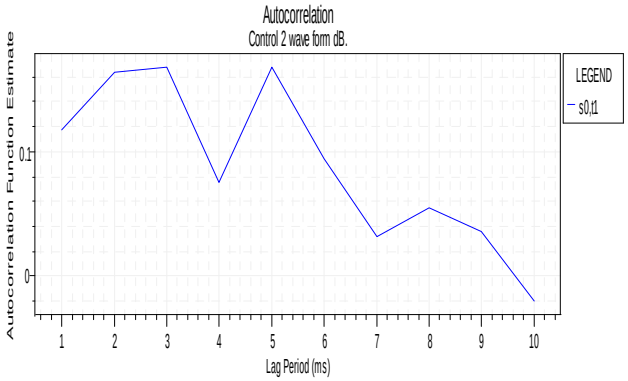

Figure 5A Tracing of spike train and peak patterns representing electrode placements and recordings for the Control in form of an Autocorrelation Function Estimate graph. The maximum peak for the control is 0.16 was reached twice and upon stimulation at 4ms, the response then subsided as stimulus was withdrawn. Compare with the treatment group.

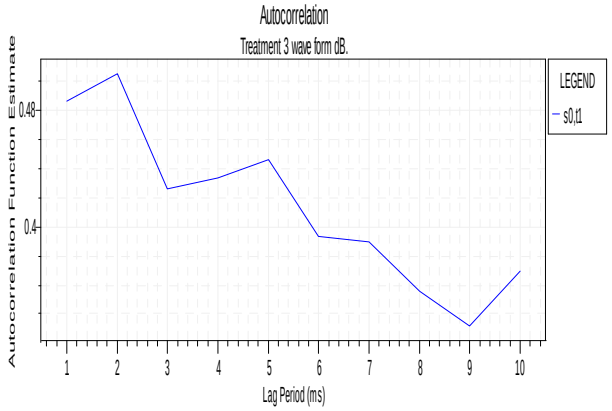

Figure 5B Tracing of spike train and peak patterns representing electrode placements and recordings for the Control in form of an Autocorrelation Function Estimate graph. The maximum peak for the treatment is 0.50. Upon stimulation at 3ms, the maximum response peak was 0.44 then subsided as stimulus was withdrawn. Compare with the sharp response in the control group.

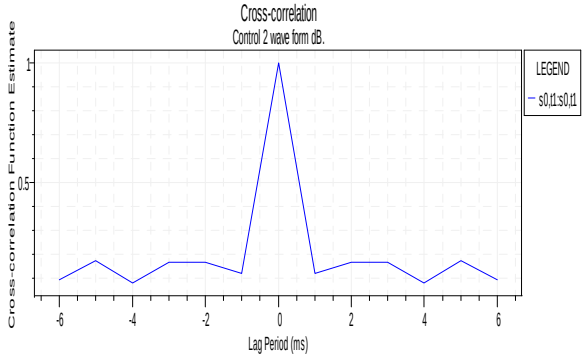

Figure 6A Tracing of spike train and peak patterns representing electrode placements and recordings for the treatment in form of a Cross-correlation Function Estimate graph. The maximum peak for the control is 1.0.

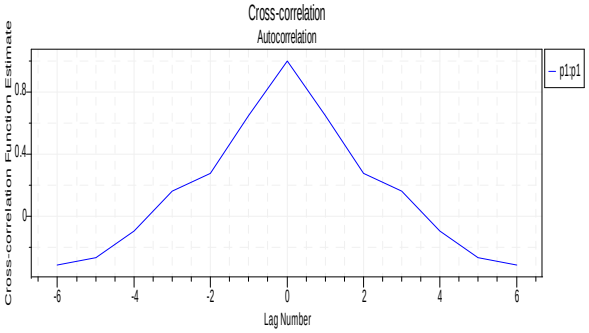

Figure 6B Tracing of spike train and peak patterns representing electrode placements and recordings for the treatment in form of a Cross-correlation Function Estimate graph. The maximum peak forthe treatment is 0.9. Notice the wave patterns in the treatment, compare with control.

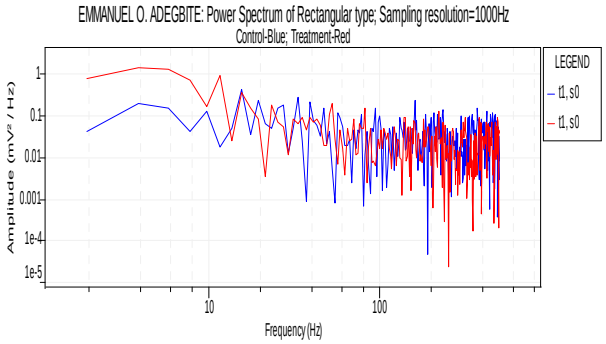

Figure 7 A power spectrum of rectangular type at a sampling resolution of 1000Hz showing the wave patterns in the control (Blue) and the treatment (Red). Result depicts that even in the absence of stimulus, the response still lasted for longer time in the treated group but a sharper response was observed in the control group.

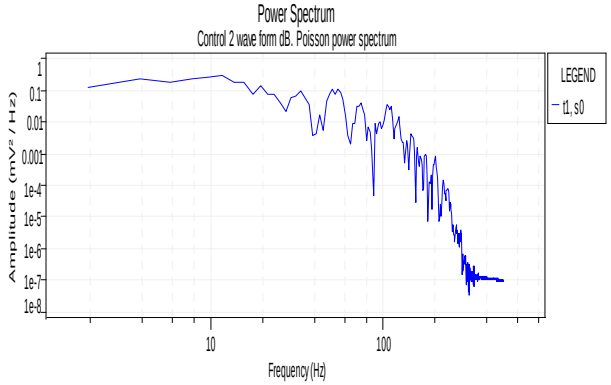

Figure 8A The response rate and firing rate with action potential represented by the Poisson power spectrum. There is maximum firing in the control and subsided as stimulus was removed. Readings taken when electrodes were placed in the motor cortex.

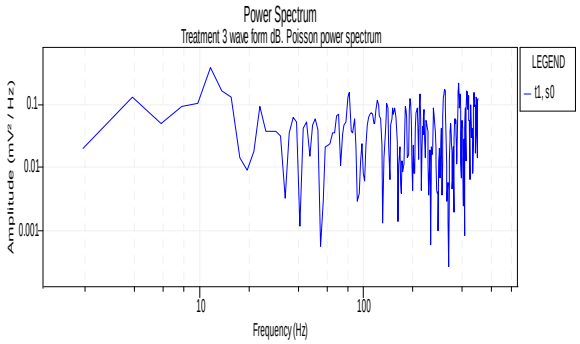

Figure 8B The response rate and firing rate with action potential represented by the Poisson power spectrum. The result also shows reduced firing rates in the treated group at these intervals, but prolonged Action Potential when compared with the control.

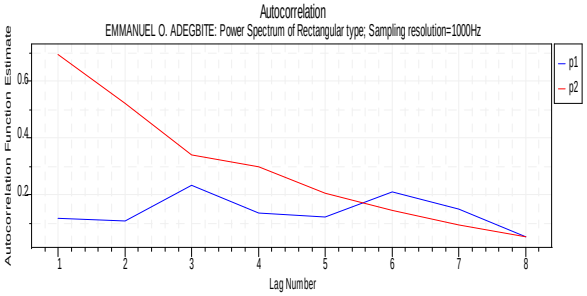

Figure 9 Tracing of spike train and peak patterns representing electrode placements and recordings for the control (Red) and the treatment (Blue) represented in a power spectrum of rectangular type at a sampling resolution of 1,000Hz. The maximum peak for the control is 0.7 and that of the treatment is 0.2. The result shows reduced firing rate in the treated group.

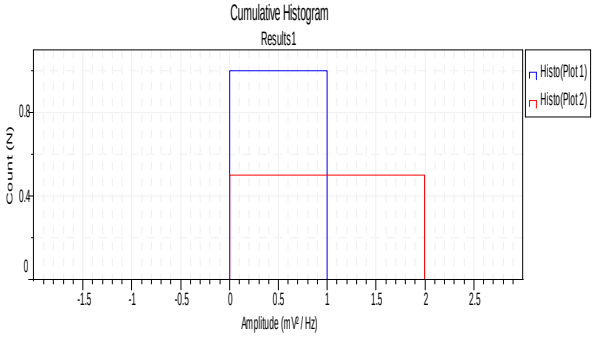

Figure 10 Tracing of spike train and peak patterns representing electrode placements and recordings for the control (Blue) and the treatment (Red) in form of a conventional cumulative histogram. The maximum peak for the control is 1.0 and that of the treatment is 0.5. Also, the amplitude of control spans over 1 mV^2/Hz while that of the treatment spans over 2 mV^2/Hz.

Neurobehavioural Assessment Tests

Introduction

The types of Neurobehavioural Assessment Tests performed were Open-field Exploration test, Parallel bar test, Rotarod test, Clasping, Grooming, Spontaneous, and Locomotor activities.

Analogousvariations in physiological indicators and behavioural responses to fear and painful stimuli in humans and other animals suggest the possibility of homologous or analogous, ethologically motivated defensive responses.[23] In

[2]Blanchard RJ, Griebel G, Henrie JA, Blanchard DC. Differentiation of anxiolytic and panicolytic drugs by effects on rat and mouse defense test batteries.Neurosci.Biobehav. Rev. 1997;21(6):783–789. [PubMed]

the account of human anxiety disorders, the model of "state" and "trait"anxiety have a long history. However, recently, these concepts have been proposed as means of differentiating situational anxiety-like behaviour in mice from anxiety that transcends the situation and is an enduring condition in the animals.[4]

Open-field test: The test provides a unique opportunity to systematically assess novel environment exploration, general locomotor activity, and provide an initial screen for anxiety-related behaviour in mice.

Parallel bar test: Two parallel steel bars 1 m in length and 4 mm in diameter were fixed 30 mm (on their centers) apart by wooden supporting columns at their ends. The bars were 60 cm above the floor.

The most commonly used measure of overall exploratory/locomotor activity is currently the total distance traveled. Although horizontal activity appears to be recording a similar measure, in fact, the equipment records every beam break including those not associated with ambulatory activity (e.g., repetitive head movements).In contrast, the calculation of total distance includes constraints that exclude units of activity that are generated by these repetitive beam breaks. Time spent investigating the central region of the chamber can be reported as a percent of total session length for both the short and longer habituation versions of this test.

METHODOLOGY AND EXPERIMENTAL DESIGN

A cohort study was performed with 10 animals in the control group and 10 animals in the treatment group.

Treatment duration and Dosage

The animals were dosed at $LD_{50/14}$ over a period of two weeks at about 9am daily.

For open field analyses, mice were evaluated for 3 minutes in an empty sterile home cage.

Rotarod- Rotarod ability was assessed on an accelerating Rota-Rod. The mice had a training run and then were tested. The rotarod accelerated from 3 to 30 rpms over a 3-min period. Latency to fall was recorded in rpms and seconds. Locomotor activity- The investigator counted the number of times the mice touched the sides of the cage over the 2-min period. The mouse had to put at least one paw on the side and place its paws back on the floor before another touch was counted. The actual number of touches was recorded.

Statistical Analysis

Neurobehavioural Assessment

Tremor: Mice were evaluated for presence or absence of tremors. None or mild were scored as 1 (normal) and marked was scored as 2 (abnormal).

1=None/Mild
2= Marked

Test	Control (mean±sd)	Treatment (mean±sd)	P Value	Significance level
Rotarod	14.60±2.46	7.60±1.43	<0.000001	=0.05
Parallel bar test	1.68±0.27	2.97±0.82	<0.001	=0.01
Open-field test	7.00±1.70	18.7±3.20	<0.001	=0.05
Tremor	None	Moderate	<0.001	=0.05
Clasping	Forepaw tuck	Hind limb clasp	<0.001	=0.05

Spontaneous Activity: Mice were evaluated for their activity level while exploring the cage for the 2-min period. The cage floor was divided into quadrants and their exploratory movements were scored. Mice were scored 1 for moderate, covering all quadrants (normal), 2 for slow, covering 1 to 3 quadrants (still within normal behavior range), and 3 for either none or darting (abnormal).

1. Moderate: covers all quadrants
2. Slow: covers 1 to 6 quadrants
3. None or darting/circling

Clasping

Mice were suspended by the tail for 1 min. In this position, the control mice perform a swimming motion with rapidly moving paws and an arched back. The treated group mice often progress from forepaw tucking to hind and forepaw clasping with a quick release, to a complete clasp where they remain in a ball.

The mice were given a score of 1 for swimming motion or forepaw tuck (normal), or 2 for clasp and release or clasp and hold (abnormal).

1.Grooming - During a 3-min observation, occasional, slow, or intermittent grooming is normal mouse activity. Absence of grooming or, as in the case of the treatment group, excessive and repetitive grooming is abnormal. Mice were given a score of 1 for slow or occasional (normal) and 2 for excessive (abnormal), 3 for none.

Animal Group	Tremor	Clasping	Spontaneity	Grooming
CONTROL	**1**	**1**	**1**	**1**
TREATMENT	**2**	**2**	**3**	**2**

CONCLUSION

We conclude that: on the basis of the data, the mean revolution per minute is lesser for the control than the treatment population for the RotarodNeurobehavioural test.

We reject H_o and conclude that the mean population for the parallel bar test is lesser for the controls than for the treatment subjects for the parallel bar test, and upon placement on the bars, the treated group mice seem confused and anxious and it took them average of 2-3 minutes unlike the control animals to move. Also, on the average, the treated animals took more tha 180 seconds to get to the edge of the rods.

Upon assessment of their general locomotor activity and anxiety-related behaviours, it can thus be inferred that the treatment showed greater degree fear and anxiety than the animals in the control group. This relates to possible damage at the level of the hippocampal formation in the limbic system when exposed to the compound.

[3]Rodgers RJ, Cao BJ, Dalvi A, Holmes A. Animal models of anxiety: An ethological perspective. Brazilian Journal of Medical and Biological Research.1997;30(3):289–304. [PubMed]
[4]Belzung C, Griebel G. Measuring normal and pathological anxiety-like behaviour in mice: A review. Behavioural Brain Research.2001;125:141–149. [PubMed]

From the grooming test results, it can be said that there is greater tendency of irrational behaviours in the treated group (consisting of 10 animals) due to their darting or circling movements during the spontaneity test.

On the basis of Electrophysiological studies performed, it can be concluded that the functions of motor cortex are greatly compromised in acute neurotoxicity with regal dry gin as graphs plotted show slow initiation and blunt response to withdrawal or absence of stimuli following initiation when the animals were tapped during the process. This further suggests damage to the motor circuit and the cortico-thalamic system.
Future research may then look more specifically into the degree of damage done to the basal ganglia thalamo-cortical system as well as the cerebellar nuclei involved in motor functions.

Acknowledgement

Firstly, I want to thank God Almighty for making this work a success. He is the sole source of my inspiration and motivation.

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