Influence of Chronic Ethanol and Caffeine on the Neuronal Cell Density and Biochemical Quantification of Cerebral Cortex of Adult Male Wistar Rats

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ABSTRACT

The ingestion of high doses of caffeine in combo with ethanol so as to reduce the intoxicating effects of the alcohol is a very common practice in the society today. This practice is one of the contributing factor to the health challenges ravaging the populace. However, there is a little or no knowledge on the effect that the simultaneous misuse of these drinks has on the function of the. Hence this study aimed to examine the influence or how the chronic use of caffeine and ethanol can affect the neuronal cell density and biochemical quantification in the cerebral cortex of male Wistar rats.

Fifty (50) males Wistar rats with average weight ranging between 120 - 150grams were randomly divided into five groups (n=10); Control groups C1, C2 and Treatment groups T1, T2 and T3. The control groups C1 and C2 were given distilled water and 2% sucrose solution respectively while Treatment groups T1, T2 and T3 were given 10 mg/kg body weight (bw) of caffeine in 2% sucrose solution, 25% ethanol in 2% sucrose solution and 10 mg/kg body weight caffeine dissolved in 25% ethanol in 2% sucrose solution respectively. Rats were fed with standard rat chow and drinking water was provided ad libitum for 7 weeks. The rats were sacrificed by cervical dislocation and the cerebral cortex of the brain specimens were processed for biochemical protocol. Results were presented as mean ± SEM and analyse using SPSS Version 12, one-way Anova was use and level of significance was set at $P< 0.05$.

Results showed that exposure to 25% of ethanol and 10 mg/kg bw of caffeine for 7 weeks led to significant neuronal cell death to about 49 count. Biochemical analysis study for LDH showed significantly ($P< 0.012$, 0.015 and 0.03) decreased value for LDH in Treatment Group T1, T2 and T3 compared to control Groups C1 and C2. The values obtained for SDH also showed significantly ($P< 0.032$,0.038 and 0.04) decreased value for Treatment Group T1 and T2 & T3 compared to control Groups C1 and C2. The values recorded for G-6PD showed significantly ($P< 0.024$, 0.026 and 0.036) decreased for Treatment Group T1 and T2 & T3 compared to control Groups C1 and C2 respectively.

It is concluded in this research that caffeine and ethanol have neuro-generative defect to neuronal cells in the brain which could result to memory lost and other neuronal imbalanced.

Keywords: Ingestion, Caffeine, Ethanol, Cerebral cortex, Neurodegeneration, Intoxication, Misuse, Combo.

INTRODUCTION

Ethanol and caffeine is a popular and the most consumed psychotropic drugs or substance globally (1). In recent times, it has become common to ingest high doses of caffeine in combo with ethanol so as to reduce the intoxicating effects of the alcohol (2, 3, 4).

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Chronic ethanol exposure has greater negative influence on society, such as failed connections, loss of employment, psychiatric symptoms, overt neurotoxicity, liver failure, and severe cognitive dislocation etc. (5). These habitual problems consume considerable coffers for psychiatric care, organ transplants, and long- term medical treatment (6). Ethanol is one of the most generally abused substance worldwide (1). Although environmental and inheritable factors contribute to the etiology of alcohol use diseases. still, ethanol's conduct in the brain explain acute ethanol- related behavioral changes, (e.g goad followed by depressant goods) and habitual changes in geste, (e.g escalated use, forbearance, obsessive dogging, and dependence) (6).

Caffeine, the principal alkaloid in coffee, tea, and energy drinks, is one of the substances in the most widely consumed psychostimulant in the world (7), and has been widely implicated as a cognitive modulator. The primary effect of caffeine in the central nervous system is inhibition of adenosine receptors and subsequent modulation of neurotransmitter release (8). Adenosine, a neuro-modulatory signaling molecule, is normally present in the brain, and when it accumulates e.g. during increased neuronal firing, it causes a progressive decrease in neuronal activity when bound to adenosine receptors. Caffeine counters this effect by acting as an antagonist at the adenosine receptors A_1 and A_{2A} (9).

MATERIAL AND METHOD

Experimental Animals

Fifty (50) adult males Wistar rats with average weight between 120 -150g were used for this study. The rats were acclimatized for the period of two weeks under standard laboratory conditions at Animal Holding of Anatomy Department, Ladoke Akintola University of Technology, Ogbomoso, Nigeria and treated in accordance with the Guide for Care and Use of Laboratory animals prepared and compiled by the National Academy of Science and published by the National Institute of Health (1985).

Experimental design

The rats were randomly grouped into five (C1, C2, T1, T2 & T3) of ten rats in each group after acclimatization. And the duration of the treatment lasted for 7 weeks (49 days).

Table 1: Shows the experimental design

Animal grouping	Number of rats	Animal induction and treatment
Group C1 (Control group 1)	10	distilled water
Group C2 (Control group 2)	10	2% sucrose solution
Group T1 (Treated group 1)	10	10mg/kg caffeine in 2% sucrose solution
Group T2 (Treated group 2)	10	25% ethanol in 2% sucrose solution
Group T3 (Treated group 3)	10	10mg/kg caffeine dissolved in 25% ethanol in 2% sucrose solution

Collection of organs

The final body weights were taken and documented at the end of 7 weeks (49 days) of administration. The next day at 8.00 HRS GMT, all the rats were sacrificed by cervical dislocation. The cerebral cortex of the brain specimens was harvested, weighed, documented and immediately transferred to fixative (10% formol calcium) for routine histological paraffin embedding procedures and stained for Nissls substance using Cresyl violet as described by Venero et al. (10).

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Tissue Processing

The section was produced by normal routine histological methods of fixation, dehydration, impregnation, embedding, sectioning and staining with Nissls substance using Cresyl violet by Venero et al., (10). The micrographs of the relevant stained sections were subsequently taken with the aid of a light microscope.

Statistical analysis

All the data were analyzed by analysis of variance (ANOVA) and post-hoc tests (Tukey HSD) used to determine source of a significant effect. Results are expressed as Mean ± S.E.M., $p<0.05$ is taken as accepted level of significant difference from vehicle or standard.

RESULT AND DISCUSSION

Results

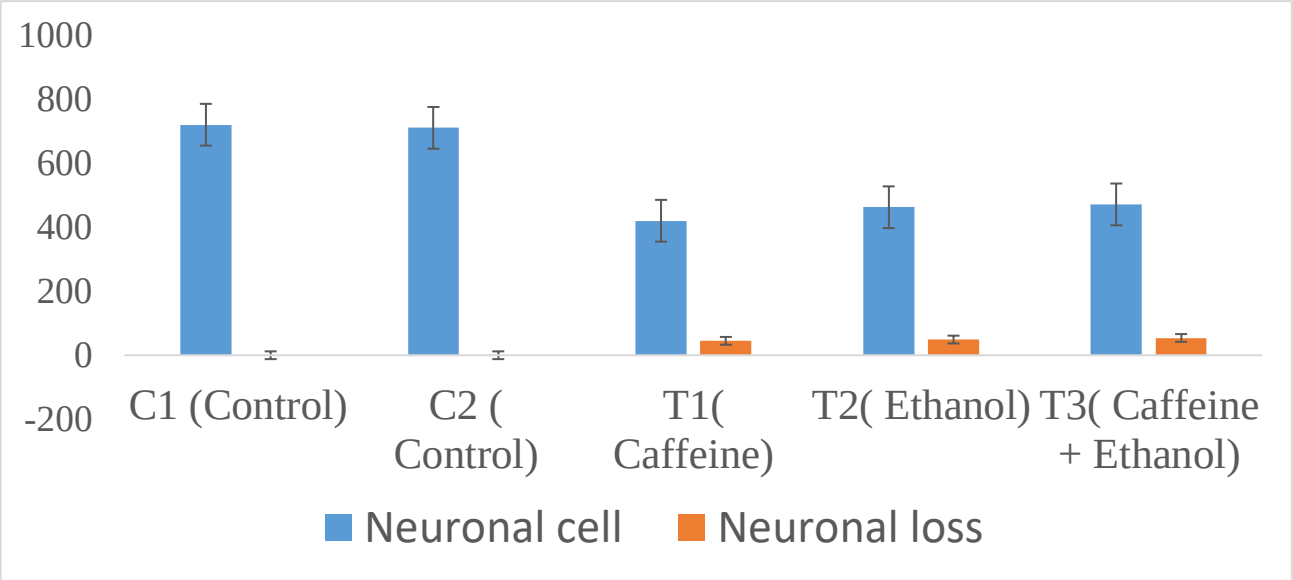

Figure 1: Histogram representation of the neuronal cell density and percentage neuronal loss following the administration of ethanol and caffeine.

Table 2: Showed Biochemical Quantification analysis following the administration of ethanol and caffeine. Data were expressed as Mean ± S.E.M.

GROUPS	LDH (IU/L)	SDH (IU/L)	G6PDH (IU/L)
Control Group C1 (Distilled water)	1507.61 ± 9.10	1296.76± 6.18	1626.41± 4.48
Control Group C2 (2% Sucrose solution)	1520.51± 8.43	1306.38± 5.38	1636.41± 4. 10
Group T1 (10mg/kg of Caffeine in 2% Sucrose solution)	1205.15 ± 4.20*	1177.19± 5.21*	1245.49± 3.22*

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Group T2 (25% Ethanol in 2% Sucrose solution)	1080.05± 3.89*	992.38± 9.38*	692.59± 2.38*
Group T3 (10mg/kg Caffeine in 25% Ethanol in 2% Sucrose solution)	993.61± 3.12*	794.76± 6.18*	526.41± 2.05*

Significance: The result obtained by comparing Group C1 and C2 to other Group (T1, T2 and T3), shows low in biochemical parameters. $P < 0.05$, values less than 0.05 are considered significant (*).

Discussion

Influence of Ethanol and caffeine on neuronal cell density

Caffeine is a popular psychostimulant, which is frequently consumed with ethanol (1). However, generalized toxic effects of ethanol on the brain have been widely demonstrated as impairing neurogenesis and promoting neuronal cell death (6).

Cell viability results showed that exposure to 25% of ethanol for 7 weeks led to significant neuronal cell death to about 49 count. The length of 7 weeks chosen here for ethanol exposure represented a sub-chronic ethanol abuse. For caffeine, cell viability significantly decreased to about 45 at 7 weeks of exposure to 10mg/kg of caffeine. At this dose and length of caffeine exposure, a previous study shows that it triggers autophagy by promoting, a degradation pathway by which the cell digests its own components (11).

Influence of Ethanol and caffeine on Biochemical quantification

Ethanol and caffeine were independently found to decrease in the activity of LDH, SDH and G6PD in treated group. Ethanol-caffeine, produced a remarkable decrease in the activity of Na+-K+-ATPase. These effects could be attributed to the perturbation of the ionic equilibrium of the nerve receptors. The results showed that Na+-K+-ATPase, which maintains ionic homeostasis in the brain was inhibited by ethanol- caffeine nut interaction. The major effect was the perturbation of the ionic equilibrium of the nerve receptors during which the Na+-K+-ATPase was essentially by-passed with the showed that Na+-K+-ATPase, which maintains ionic homeostasis in the brain was inhibited by ethanol- caffeine nut interaction (12). The major effect was the perturbation of the ionic equilibrium of the nerve receptors during which the Na+-K+-ATPase was essentially by-passed with the results that K^+ ion very rapidly left the brain nerve cells, resulting in a decrease in ATP production, and ionic transport, hence reduced activity of Na+-_K+-ATPase (13)

CONCLUSION

The compilation of the results gotten from these research showed that caffeine and ethanol have neuro-generative defect to neuronal cells in the brain which could result to memory lost and other neuronal imbalanced. Further studies on the mechanism of neuronal cell defect from the combined drinks of ethanol and caffeine is therefore recommended.

DECLARATIONS

Ethics approval

The animals used were in accordance with the rules and guidance of Ladoke Akintola University of Technology, Faculty of Basic Medical Sciences, Ogbomosho, Oyo State, Nigeria

Data Availability Request

The data generated during the study will be provided on a reasonable request from corresponding author.

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Declaration of interests Statement

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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