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EFFECT OF ALCOHOL AND KOLANUT INTERACTION ON BIOCHEMICAL INDICES OF NEURONAL GENE EXPRESSION IN WISTAR ALBINO RATS

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Summary: Effect of alcohol and kolanut interactions on biochemical indices of neuronal gene expression in Wistar albino rats was studied. Thirty Wistar albino rats were divided into six groups of five (5) rats per group. The control group (1) received via oral route a placebo (4ml of distilled water). Groups 2 - 6 were treated for a period of 21-days with (10% v/v) 50mg/kg body weight of alcohol, 50mg/kg body weight of kolanut, 50mg/kg body weight of caffeine, 50mg/kg body weight of alcohol and 50mg/kg body weight of kolanut, and 50mg/kg body weight of alcohol and 50mg/kg body weight of caffeine in 4.0ml of the vehicle via gastric intubation respectively. One day after the final exposure, the brain of each rat was harvested and processed to examine several biochemical parameters, i.e., total protein, DNA, RNA and protein/RNA ratios. The status of neuronal gene expression was monitored through assessment of these parameters. The results showed that alcohol-kolanut co-administration decreased brain total protein, DNA, RNA levels and protein/RNA ratios, and inhibited gene expression. These effects, in turn, inhibited DNA transcription, MRNA splicing and protein synthesis, and polypeptide expression, which are necessary for the growth, development, differentiation and cell survival.

Key Words: *Alcohol, Kolanut, Gene expression, DNA, RNA, Protein.*

Introduction

Alcohol and Kolanuts are common items of entertainment in community functions. Kolanut contains constituents, which include kolanin, quinine, caffeine, theobromine and theophylline (Adeyeye and Ayejuyo, 1994; Eteng *et al*, 1992; Abulude, 2004; Obochi, 2006). These constituents are also constituents of coffee, cocoa and tea leaves and are widely consumed through their beverages such as snacks (coke, schwepps, bitter lemon, etc), pharmaceutical products, over the counter drugs, and extracts of coffee, cocoa and kolanuts (Eteng, *et al*, 1997; Abulude, 2004; Obochi, 2006). Alcohol is widely consumed through alcoholic beverages such as table wines, beers, desert or cocktail wines, cordials, liquors, whisky and brandy. These beverages (alcohol and kolanuts) are valued as foods, medicine and ceremonial drinks. Although, negligibly nourished, alcohol is an energy producing food like sugar (El-mas *et al*, 1994; Dorhman *et al*, 1997; Fadda & Rossetti, 1998; Koobs *et al*, 1998; Lieber, 1999; 2000; and Danbolt, 2001). These drugs (alcohol and kolanuts) have opposing effects on the brain. Brain function involves subtle chemical and electrical processes, which can easily be altered and modified with the use of psychoactive drugs (Obochi, 2006).

Gene expression is linked with an interplay of neurotransmitter uptake during DNA transcription, mRNA splicing and degradation together with protein synthesis, and polypeptide expression. Organisms, including humans adapt to environmental changes by altering gene expression. The regulation of the expression of the genes is necessary for the growth, development, differentiation and the very existence of the organism. The process of alteration of gene expression involves the interaction of specific binding proteins such as nerve growth factors, glial derived neurotrophic factors, peptides, etc, with various regions of DNA in the immediate vicinity of the transcription site (Obochi, 2006). The composite of these induced changes in gene expression may result in the cellular responses to tolerance and dependence and may lead to neuronal dysfunction. Thus, the metabolic

interaction between alcohol and kolanut may be of medical importance for diagnosis and or treatment of neuronal disorders.

Materials and Methods

Experimental Animals

Thirty (30) Wistar albino rats weighing between 150 –280g obtained from the disease free stock of the animal house, Department of Biochemistry, College of Medical Sciences, University of Calabar, Nigeria were used for the study. The animals were randomly assigned into six (6) groups of five (5) animals per group. Each rat in a study group was individually housed in a stainless cage with plastic bottom grid and a wire screen top. The animal room was adequately ventilated, and kept at room temperature and relative humidity of 29+ 2^0c and 40-70% respectively with 12 hour natural light-dark cycle. The animals were fed ad libitum with water and rat chow (livestock feeds Ltd, Calabar, Nigeria). Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from cages daily.

Treatment Regimen

The control group (1) received via oral route (oral gavage) a placebo (4ml of distilled water). Groups 2 to 6 were treated for a 21-day period with (10% v/v) 50mg/kg body weight of alcohol, 50mg/kg body weight of kolanut, 50mg/kg body weight of caffeine, 50mg/kg body weight of alcohol and 50mg/kg body weight of kolanut, and 50mg/kg body weight of alcohol and 50mg/kg body weight of caffeine in 4.0ml of the vehicle via gastric intubation (ie, orally using orogastric tubes and syringes) respectively. The experiments were conducted between the hours of 9.00am and 10.00am daily.

Preparation of Caffeine

Synthetic caffeine was obtained from May and Baker (M&B) limited, Enfield, Middle Sex, United Kingdom, and used for the study. A stock solution of caffeine was prepared by dissolving 20g of powder caffeine in 500ml of hot distilled water. The solution was allowed to cool to room temperature, and 50mg/kg body weight of caffeine was administered to groups 4 and 6 in 4.0ml of the vehicle via gastric intubation.

Preparation of Kolanut:

Kolanuts were obtained from the Bogobiri market, Calabar, Nigeria and used for the study. The kolanuts were washed, an dried at 60^0c for 12 hours, and ground using electric kenwood blender. 20g of the kolanut was dissolved in 500ml of hot distilled water. Out of the stock solution prepared 50mg/kg body weight was administered to the animals in groups 3 and 5 in 4.0ml of the vehicle via gastric incubation.

Preparation of Alcohol

The alcohol used was distilled from palm wine (Elias guinensis) using quick fit distillation apparatus. 10% v/v of the alcohol was prepared and 50mg/kg body weight of alcohol was administered to the animals in groups 2,5 and 6 in 4.0ml of the vehicle via gastric incubation.

Sample Preparation

One day after the final exposure, the animals were anaestheticized by inhalation of an over dose of chloroform, and the brain of each rat was harvested, ground using mortar and pistle, and buffered with TRIS-HCL buffer, pH 7.4. A whole homogenate (WH) was prepared by centrifugation (4000xg, 30 minutes). The supernatant was again centrifuged at 6000xg for 20 minutes and made up to 100ml mark with the TRIS-HCL buffer, pH 7.4 in a volumetric flask. The whole homogenate thus obtained was stored at -70% in the freezer and used for the various assays.

Biochemical Assays:

The whole homogenate (WH) obtained was used for the analysis of brain total protein, DNA and RNA levels. Brain protein/RNA ratios were calculated. Brain total protein was determined by the Biuret method described by Lowry *et al* (1951), which represents the modifications of Gornall *et al* (1949). DNA and RNA were determined by the Diphenylamine and Orcinol assays respectively described by Burton (1956). Brain protein/RNA ratios were calculated.

In protein analysis, 1.5ml of the whole homogenate was measured and 0.2ml of 5% sodium deoxycholate (Doc) in 0.01 N KOH was added, and mixed. Then, 1.5ml of the Biuret reagent (1.5og $CuSo_4$.$5H_2O$, 6.0g sodium potassium tartrate, and 300ml of 10% NaoH per L) was added and mixed. The tubes were incubated for 15 min at 37^0c and the absorbances were read at 540nm against a reaction blank in a spectrophotometer.

In DNA analysis (Diphenylamine assay), 2.0ml of the whole homogenate (WH) was measured and 2.0ml of the diphenylamine reagent (Dissolved 0.7g of diphenylamine in 50ml of glacial acetic acid and 0.75ml of conc. H_2So_4 was added.

Just prior to use, 0.25ml of cold 1.6% acetaldehyde was added. [Prepared in a fume hoods]) the tubes were allowed to cool to room temperature and the absorbances were read at 600nm against a reaction blank.

In RNA analysis (Orcinol assay), 0.5ml of the whole homogenate (WH) was measured and made up to 2.0ml with 5% trichloroacetic acid (TCA). Then 2.0ml of orcinol reagent (Dissolved 0.5g of orcinol in 50ml of 0.1% $Fecl_3$ in conc. Hcl. [prepared in a fume hood]) was added and mixed. The orcinol reagent was prepared just prior to use. The tubes were heated in a boiling water bath for 15 minutes. The tubes were removed and allowed to cool to room temperature and the absorbances were read at 640nm against a reaction blank in a spectrophotometer.

Statistical Analysis

Data collected were expressed as mean $\pm$ standard deviation (SD), analysis of variance (ANOVA) and the student 't' test were used for analysis. Values of $p<0.05$ were regarded as significant.

Results

Table 1 presents the results of the effects of the treatment on brain total protein, DNA, RNA and Protein/RNA ratio levels in wistar albino rats. The results showed that kolanut and caffeine independently produced a non significant increase ($P<0.05$) in values of the total protein, DNA, RNA and Protein/RNA ratios relative to control while alcohol had a contrary effect. However, co-administration of alcohol and kolanut as well as alcohol and caffeine produced a significant decrease ($P<0.05$) in values of the total protein, DNA, RNA and Protein/RNA ratios relative to control. These results showed that alcohol suppressed the effects of kolanut and caffeine.

Table 1: Effect of treatment on brain total protein, DNA, RNA and Protein/RNA ratio levels in wistar lbino rats.

		Parameters			
	Group (N)	Brain total protein (mg/ml)	Brain total DNA (mgDNA/ml)	Brain total RNA (mgRNA/ml)	Brain Protein/RNA ratio
1.	Control	9.83±0.63	5.39±0.88	6.65±0.48	1.48±1.31
2.	Alcohol	6.74±0.48*	4.89±0.41*	6.34±0.35*	1.06±1.37*
3.	Kolanut	12.46±0.57*	8.93±0.69*	6.54±0.75*	1.90±0.76*
4.	Caffeine	14.25±0.62*	10.28±0.87*	6.24±0.78*	2.28±0.80*
5.	Alcohol-Kolanut	7.48±0.63*	5.27±0.67*	6.26±0.43*	1.20±2.86*
6.	Alcohol-Caffeine	8.48±0.67*	5.86±0.58*	6.38±0.41*	1.30±1.46*

** Significantly different from control, P<0.05 using ANOVA and student 't' test.*
Values are expressed as mean ± SD, N = Number of rats per group = 10

Discussion

In this study, kolanut and caffeine were independently found to increase total protein, DNA and RNA levels and Protein/RNA ratio while alcohol, alcohol-kolanut and alcohol-caffeine decreased total protein, DNA and RNA levels and Protein/RNA ratio. The results showed that kolanut acted synergistically with alcohol to decrease total protein, DNA and RNA levels, and Protein/RNA ratio. The results of this study agreed with earlier studies of Mackler and Eberwine (1991), Snyder *et al* (1992), Baek *et al* (1994), Kim *et al* (1996), Bonner *et al* (1996), Miller (1996) and McAlhany *et al* (2000). The reports of these workers showed that the depressant actions of alcohol interfered with synthetic processes hence a reduction in total protein, DNA and RNA, with the overall effect on the reduction in the cell number and neurophil volume, alteration of myelin formation due to interference with protein synthesis. This could suggest that the mechanism of alteration in rat brain protein synthesis might involve interaction of the brain polysomes with alcohol, with a resultant alteration in messenger RNA components associated with the ribosomal units, which are used for protein biosynthesis. The overall effect was the decrease in Protein/RNA ratio, an index of gene expression (Schafer *et al* 2001). The process of alteration of gene expression involves the interaction of specific binding proteins with the various regions of DNA in the vicinity of the transcription site and

this produces either a positive or negative effect of transcription (Snyder *et al*, 1992; Keeton *et al*, 1993; Johnson and Barford, 1993; Granner, 1996). This could be attributed to the suppression of the metabolic processes by alcohol, thereby inhibiting gene transcription signal transduction and protein synthetic pathways, in parts, through competition for a common microsomal detoxification process due to the interference of alternative pathways (mostly the microsomal ethanol oxidizing systems - MEOS) (Snyder *et al*, 1992; Phung and Black, 1999; Lieber, 1999; 2000; Lindgreen, 2001; Danbolt, 2001; Obochi, 2006).

The neurodegenerative actions of alcohol as expressed in depletion of total protein, DNA and RNA levels might be derived from its reduction of available nerve growth factor receptors, which were responsible for the cell survival, development and differentiation, resulting in the modulation of neurotransmitter uptake at the level of DNA transcription, mRNA splicing and degradation together with protein synthesis (Baek *et al*, 1994; Dohrman *et al*, 1997; Fadda and Ropssetti, 1998; elman *et al*, 1999; Heaton *et al*, 2000; Schafer *et al*, 2001).

Conclusively, alcohol - kolanut interactions depressed neuronal function and inhibited gene expression, leading to an impairment in growth, development, differentiation and could potentiate a risk to tolerance and dependence.

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