

EFFECT OF ALCOHOL AND KOLANUT-CO-ADMINISTRATION ON SERUM AND BRAIN CHOLESTEROL AND PHOSPHOLIPID LEVELS IN WISTAR ALBINO RATS

M. OBI-ABANG, A. E. ABARA, G. O. OBOCHI, S. P. MALU, G. A. BASSEY, E. O. EFFIOM AND I. E. IBOR

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ABSTRACT

Effect of alcohol and kolanut co-administration on serum and brain total cholesterol and phospholipids levels 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 (4.0ml of distilled water). Groups 2 through 6 were treated for 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 respectively. One day after the final exposure, the brain and blood of each animal were harvested and processed to examine serum and brain total cholesterol and phospholipids. The results showed that alcohol and kolanut co-administration increased serum and brain total cholesterol and phospholipids, which altered cholesterol and homeostasis disruption of and phospholipase-signaling pathway, and lipid trafficking within neuronal cells, and could trigger cardiovascular and neuronal diseases.

KEYWORDS: Alcohol, Kolanut, Cholesterol and Phospholipids

INTRODUCTION

Alcohol and Kolanuts are common items of entertainment in community functions. Kolanut contains constituents, kolanin, quinine, caffeine, theobromine and theophylline (Adeyeye and Ayejuyo, 1994; Eteng et al., 1997; Abulude, 2004; Obochi, 2006). These constituents are also constituents of coffee, cocoa, bean seeds and tea leaves and are widely consumed through their beverages such as snacks (coke, schwepps, bitter lemon), pharmaceutical products, over the counter drugs, and extracts of coffee, cocoa, tea and kolanuts. (Adeyeye and Ayejuyo, 1994; 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 are valued as food, medicine and ceremonial drinks. Although, negligible, alcohol is an energy producing food like sugar (Elmas 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 (Obochi, 2006), and their metabolic interactions may be of medical interest in diagnosis and or treatment of neuronal disorders.

Cholesterol, a structural component in cell membranes and plasma lipoproteins, is both absorbed from the diet and synthesized in the liver and other body tissues such as the brain. It contributes to the formation of adrenocorticoid steroids, bile salts, androgens and estrogens.

In human plasma, the main phospholipids are lecithins, cephalins and sphingomyelins, and these are structural components of the brain. Dietary phospholipids are partially broken down by pancreatic enzymes before absorption by the mucosal cells. Phospholipids fulfill a variety of body functions, including cellular membrane composition and permeability, and some control of enzyme activity within the membrane. They have a tendency to concentrate at cell membranes and aid the transport of fatty acids and lipids across the intestinal barrier, and from the liver and other fat

depots to other body tissues. Phospholipids are also essential for pulmonary gas exchange. Sphingomyelin acts as an insulator around nerve fibers (Obochi, 2006). The levels of serum and brain cholesterol and phospholipids reflect the activities of the inner and neuron.

MATERIALS AND METHODS

Experimental Animals

Thirty (3) wistar albino rats weighing between 150 – 280g obtained from the disease free stock of the animal house, Department of Biochemistry, College of Medical Science, 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 related humidity of $29 \pm 2^\circ\text{C}$ and 40 – 70% respectively with 12 hour natural light-dark cycles. 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.

Sample Preparation

One day after the final exposure, the animals were euthanized by inhalation of an over dose of chloroform. The

M. Obi-Abang, Department of Chemistry/Biochemistry, Cross River University of Technology, Calabar.

A. E. Abara, Department of Chemistry/Biochemistry, Cross River University of Technology, Calabar.

G. O. Obochi, Department of Chemistry/Biochemistry, Cross River University of Technology, Calabar.

S. P. Malu, Department of Chemistry/Biochemistry, Cross River University of Technology, Calabar.

G. A. Bassey, Department of Chemistry/Biochemistry, Cross River University of Technology, Calabar.

E. O. Effiom, Department of Chemistry/Biochemistry, Cross River University of Technology, Calabar.

I. E. Ibor, Department of Chemistry/Biochemistry, Cross River University of Technology, Calabar.

brain of each rat was harvested, ground using mortar and pestle, and buffered with TRIS-HCL buffer, pH 7.4. A whole homogenate (WH) was prepared by centrifugation (at 4000xg for 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 cholesterol and phospholipids while the serum collected (after centrifugation) was used for the analysis of serum total cholesterol and phospholipids. Serum and brain total cholesterol and phospholipids levels were determined with modifications of the method described by Bartlett (1959).

Brain cholesterol was extracted with absolute ethanol evaporated to dryness under nitrogen and the residues resuspended in ethanol. 0.1ml of the extract was pipetted using automatic micropipettor and sufficient absolute ethanol was added to bring the volume to 2.0ml. Then 2.0ml of the cholesterol reagent was added and mixed carefully at the lowest setting of the vortex mixer. Cholesterol reagent (or ferric chloride reagent) consists of 80ml of a solution of 2.5% $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in conc. H_3PO_4 and 920ml of conc. H_2SO_4 per litre. It was transferred with an automatic dispenser. The mixture was incubated for 30 minutes at room temperature (37°C), and the absorbance read at 550nm. The amount of cholesterol was determined in mg/g of tissue.

Phospholipids extract was made by digesting the total lipid extract with perchloric acid. 0.2ml of the sample was pipetted. Then 0.2ml of 10% H_2SO_4 was added, and incubated at 140°C for 1hr. 0.05ml of 30% H_2O_2 was added and incubated again at 140°C for 40 minutes, allowed to cool to room temperature, 0.05ml. Milli-Q H_2O was added and mixed thoroughly on a vortex mixer. Then 0.5ml of molybdate reagent was added and again mixed thoroughly. This reagent was prepared just prior use and was protected from light by wrapping the container with aluminium foil. The mixture was incubated for about 15 minutes at 50°C and the absorbance read at 820nm against a blank in HACH/DR/3000 spectrophotometer using 1-ml disposable plastic cuvette.

Molybdate reagent was prepared as follows:

- (1) Make up 15% ascorbic acid solution (1.5g of ascorbic acid + 10ml. milli-Q H_2O)
- (2) Combine 1.0ml of 5% ammonium molybdate + 3.0ml of 15% ascorbic acid + 6ml of milli-Q H_2O .

The principle is that phospholipids which complexes with molybdic acid was reduced by ascorbic acid to give a heteropoly blue colour.

Preparation of Caffeine

Synthetic caffeine was obtained from May and Baker (M & B) limited, Enfield, Middlesex, 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, air dried at 60°C 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 intubation.

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 intubation.

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

Tables 1 & 2 present the effects of the treatment on serum and brain total cholesterol and phospholipids respectively. The results showed that kolanut and caffeine independently decreased ($p < 0.05$) the values of both the cholesterol and phospholipids levels while alcohol, alcohol-kolanut and alcohol-caffeine co-administrations increased ($p < 0.05$) the values of both the cholesterol and phospholipids contents. The results showed that alcohol suppressed the effects of kolanut and caffeine.

Table 1: Effect of the treatment on serum and brain total cholesterol levels in wistar albino rats.

Group (N)	Serum total Phospholipid (mg/g of tissue)	Brain total Cholesterol (mg/g of tissue)
1. Control	7.43 \pm 1.45	44.31 \pm 1.69
2. Alcohol	11.27 \pm 1.73*	7.86 \pm 1.48*
3. Kolanut	5.93 \pm 1.37*	3.21 \pm 1.34*
4. Caffeine	6.19 \pm 1.43*	3.79 \pm 1.43*
5. Alcohol-Kolanut	10.31 \pm 1.69*	5.47 \pm 1.87*
6. Alcohol-Caffeine	9.48 \pm 1.74*	5.16 \pm 1.73*

* Significantly differently from control, $P < 0.05$ using ANOVA and student 't' test.

Value are expressed as mean \pm SD.

N = Number of rats per group = 10

Table 2: Effect of the treatment on serum and brain total phospholipids levels in wistar albino rats.

Group (N)	Serum total Phospholipid (mg/g of tissue)	Brain total Cholesterol (mg/g of tissue)
1. Control	4.28 \pm 1.47	3.38 \pm 1.38
2. Alcohol	5.20 \pm 1.53*	5.61 \pm 1.63*
3. Kolanut	3.92 \pm 1.23*	2.08 \pm 1.23*
4. Caffeine	3.31 \pm 1.23*	2.13 \pm 1.27*
5. Alcohol-Kolanut	4.59 \pm 1.52*	3.23 \pm 1.47*
6. Alcohol-Caffeine	4.36 \pm 1.51*	3.38 \pm 1.59*

* Significantly differently from control, $P < 0.05$ using ANOVA and student 't' test.

Values are expressed as mean \pm SD.

N = Number of rats per group = 10

DISCUSSION

In this study, kolanut and caffeine independently produced a decrease in the levels of the serum and brain cholesterol and Phospholipid while alcohol as well as alcohol and kolanut and alcohol and caffeine co-administration produced an increased in the levels of the serum and brain cholesterol and phospholipids. This showed that kolanut acted synergistically to increase the serum and brain cholesterol and phospholipids level. These results may suggest that kolanut and caffeine independently, may be useful in the treatment of atherosclerosis by reducing total cholesterol levels. Accumulation of serum and brain total cholesterol and Phospholipid of alcohol, alcohol-kolanut and alcohol-caffeine treated rats may involve activation of cholesterol metabolizing enzymes and phospholipases which are involved in the process of transmitting ligand-receptor induced signals from the plasma membrane to intracellular proteins. Activation of these enzymes particularly Phospholipase D and protein kinase C leads to hydrolysis of phosphatidylcholine which yields Phosphatidic acid and further hydrolysis of Phosphatidic acid results in diacylglycerol and free fatty acids. This resulted in an elevated and unregulated activation of protein kinase C, resulting in disruption of normal cellular growth and proliferation control, leading to neoplasia. This may suggest that the antiproliferative effects of alcohol-kolanut interaction in glial cells could be due to the disruption of the phospholipase-signaling pathway, and structural matrix of the neuronal membranes, which might contribute to membrane tolerance to alcohol and kolanut, resulting in alteration in the cholesterol homeostasis, and lipid trafficking within neuronal cells. This could trigger cardiovascular and neuronal diseases.

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