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Effect of Alcohol-Kolanut Interaction on Brain Metabolism in Wistar Albino Rats

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Abstract: Effect of alcohol-kolanut interaction on brain metabolism in wistar albino rats was studied. Total 30 wistar albino rats were divided into 6 groups. Group 1 was the control, while groups through 6 were treated with alcohol (10%v/v), kolanut extracts, caffeine, alcohol and kolanut and alcohol and caffeine respectively. The experiment lasted for 21 days. Glucose oxidase, adenylate deaminase and glutamine synthetase were assayed. The results showed that alcohol-kolanut interaction decreased glucose oxidase, adenylate deaminase and glutamine synthetase in the nervous tissue, resulting in a decreased energy (ATP) production, ionic transport and decreased neuronal processes resulting in induced apoptosis of insulin-stimulated neuronal cells, leading to brain growth retardation, DNA damage and may contribute to neuronal loss.

Key words: Alcohol, kolanut, glucose oxidase, adenylate deaminase, glutamine synthetase

INTRODUCTION

Alcohol and Kolanuts are common items of entertainment in community functions. Kolanut contains constituents, kolanin, quinine, caffeine, theobromine and theophylline. These constituents are also constituents of coffee, cocoa, bean seeds and tea leaves and are widely consumed through their beverages such as snacks (coke, schwebbs, bitter lemon), pharmaceutical products, over the counter drugs and extracts of coffee, cocoa, tea and kolanuts. Alcohol is widely consumed through alcoholic beverages such as table wines, beers, desert or cocktail wines, cordials, liqueurs, whisky and brandy. These drugs (alcohol and kolanuts) have opposing effects on the brain (Eteng *et al*., 1997; Abulude, 2004; Obochi, 2006).

Glucose oxidase catalyzes the oxidation of glucose as a major source of energy substrate for brain metabolism. Metabolism of the brain results in the production of ammonia through deamination of cyclic AMP Inosine Monophosphate (IMP) by adenylate deaminase,thus increasing the nitrogen content of the nervous tissue. Glutamine synthetase catalyzes the conversion of glutamic acid into glutamine and provides the means of transporting the ammonia away from the brain to the liver where it is converted to urea. Ammonia is derived from the amide nitrogen atom of glutamine and during the degradation of amines, purines and pyrimidines. Ammonia is a non protein nitrogen compound that helps to maintain acid-base balance through excretion of protons. Also, the body and the brain use the nitrogen fraction of ammonia to rebuild amino acids, formation of glutamine through glutamine synthetase, regulate the rate of glucolysis in the brain and in the biosynthesis of purines (Obochi, 2006).

In ammonia toxicity there is an increased membrane permeability to k+ and cl- which could interfere with electrical activity in the brain. The change in permeability arises from an increase in the proton concentration due to the increase in ammonia concentration. Thus the effect of alcohol kolanut interaction on brain energy, nitrogen and glutamate metabolism is being studied.

MATERIALS AND METHODS

Glucose oxidase was determined with modifications of the method of Trinder (1969). In this method, 0.5 mL of the brain supernatant samples was added to 4.0 mL of the glucose oxidase reagent mixed and incubated for 15 min at room temperature. 0.1 mL of 5 NHCl was then added and mixed and incubated again at least for 5 min at room temperature and the absorbance measured at 420 nm. The glucose oxidase reagent was made up as follows: Solution 1, 4.0 mg peroxidase, 500 mg of glucose oxidase in 100 mL of 0.1m sodium phosphate buffer, pH 7.0; solution 26.6 mg mL^{-1} of 0-dianiside, a reduced chromogen. The reagent was prepared by adding 1 mL of solution 2-100 mL of solution 1 just before use.

Adenylate deaminase activity was determined with modifications of the method of Atkinson and Murray (1967). The supernatant obtained after centrifugation at 2000 rpm for 20 min was subfractionated at 4000 rpm for 30 min and the clear supernatant was used for the assay. 0.1 mL of the supernatant was added to 0.35 mL to a solution containing TRIS (30nmoles), Kcl (50 nmoles) and ATP (0.1 nmoles) at 37°C. The absorbance was then measured at 365 nm in a 1mm cell in HACH/DR/3000 spectrophotometer.

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Glutamine synthetase was determined with modifications of the method of Shappiro and Stadtman (1967). In this method, the brain samples were homogenized in TRIS buffer, pH 7.4 and treated with ice cold Trichloroacetic Acid (TCA) (10%). Twenty mililiter of the protononized samples obtained on centrifugation were treated with 50 mL of a mixture made up of 2.5 g 0-pthalaldehyde and 250 mL β-mercaptoethanol in 6.5 mL methanol. After incubation at room temperature for 10 min, the samples were filtered through a 0.45 μm filter. The amounts of the metabolite, γ-glutamylhydroxamate formed after 15 min at 37°C were measured at 530 nm.

RESULTS

Table 1 and 2 present the results of the effect of the treatment on brain glucose oxidase, adenylate deaminase and glutamine synthetase activities in wistar albino rats. The results showed that there was a significant increase ($p<0.05$) in values of the glucose oxidase,adenylate deaminase and glutamine synthetase in the kolanut and caffeine treated groups relative to the controls while there was a significant decrease ($p<0.05$) in values of the glucose oxidase,adenylate deaminase and glutamine synthetase in the alcohol, alcohol-kolanut and alcohol-caffeine treated groups relative to the controls.

Table 1: Effect of the treatment on brain glucose oxidase activity

Group (N)	Glucose oxidase activity ($mmol^{-1}$ glucose)
1. Control	11.36±1.31
2. Alcohol	8.14±1.28*
3. Kolanut	13.14±1.46*
4. Caffeine	14.41±1.58*
5. Alcohol-Kolanut	8.67±1.39*
6. Alcohol-Caffeine	8.79±1.47*

* 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 = 5

Table 2: Effect of the treatment on brain adenylate deaminase and glutamine activity in wistar albino rats.

Group (N)	Adenylate deaminase ($mmol^{-1}$ Amp mg^{-1} protein)	Glutamine synthetase ($mmol^{-1}$ glutamylhydroxamate)
1. Control	8.21±0.48	9.37±0.73
2. Alcohol	5.64±0.26*	7.89±0.78*
3. Kolanut	11.67±0.53*	13.26±0.89*
4. Caffeine	13.38±0.69*	15.49±0.93*
5. Alcohol-Kolanut	5.83±0.23*	6.46±0.58*
6. Alcohol-Caffeine	6.49±0.27*	7.61±0.64*

* 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 = 5

DISCUSSION

In this study, alcohol-kolanut interaction produced a decrease in the activity of glucose oxidase, adenylate deaminase and glutamine synthetase. Kolanut independently increased their activities but its interaction with alcohol synergistically decreased their activities, resulting in decreased energy (ATP) production, ionic transport and glutamate metabolism. This could be attributed to the activation of the substrate shuttles, malate/Aspartate and glycerol phosphate shuttles by alcohol, which converted glucose 1-phosphate into fatty acids and triacylglycerol thus, increasing lipogenesis. Also reoxidation of NADH and reduction of $NADP^+$ involved electron transfer system and substrate shuttles were used to transport the hydrogen atoms into the mitochondria, which further reduced the amount of glucose available for ATP production. This resulted in decreased glucose absorption, utilization, energy (ATP) production, ionic transport and decreased neuronal processes. These effects could induce apoptosis of insulin-stimulated neuronal cells and may result in brain growth retardation. Insulin is an enzyme of glucose metabolism and is an important neurotrophic factor that stimulates differentiation-associated neurite outgrowth and also up-regulates Neuronal Thread Proteins (NTP) expression and insulin-mediated signal transduction cascade in neuronal cells by uncoupling receptor substrate-mediated insulin transduction pathway. This could result in the death of insulin-stimulated developing neuronal cells due to impaired mitochondria, DNA damage and reduced mitochondria mass, which could contribute to neuronal loss. The results however, did not adversely affect nitrogen metabolism.

CONCLUSION

The results showed that alcohol-kolanut interaction decreased glucose oxidase, adenylate deaminase and glutamine synthetase in the nervous tissue. This had led to decreased glucose absorption, utilization, energy (ATP) production and ionic transport, resulting in decreased neuronal processes leading to brain growth retardation and neuronal loss.

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