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The Influence of Alcohol Kolanut Constituents on Liver Aspartate Amino Transferase and Alanine Amino Transferase Enzyme Activities on Albino Wistar Rats

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Abstract: The effect of alcohol-kolanut (*Cola accuminata*) co-administration in dietary regimen was investigated in some liver function enzymes (Aspartate Amino transferase and Alanine amino transferase) using Standard Kits and Spectrophotometric methods. A 4:1 ratio of livestock feed and kolanut as well as 20% v/v alcohol in H₂O produced no significant change in the relative liver weights when compared to the control. However, the average body weight of the treated group were lowered (230.75±22.9 g) when compared to the control (370.50±25.17 g). In the liver homogenate (WH) and sub fractions (pms and cytosol), the activities of AST and ALT of the co-treated groups showed significant difference ($p \leq 0.05$) when compared with the control. The results indicate that the opposing effect of kolanut alcohol on the liver enzymes (AST, ALT) can be related to the neuronal function and liver function enzymes. The mechanism of the triggering effect of the constituents of alcohol kolanut on the activities of these two important liver enzymes is discussed through out this research.

Key words: Kolanut constituents, amino transferases estimations, psychoactive effects

INTRODUCTION

Alcohol beverages and their use have an ancient history valued, as foods, medicine and in ceremonials. Alcohol has been found to be a central nervous system depressant and injures the nervous system by inhibiting growth processes (Aloe *et al.*, 2000; Schafer *et al.*, 2001; Danbolt, 2001).

Kolanut on the other hand contains constituents such as kolanin, quinine, caffeine, theobromine and theophylline which are also present in coffee, cocoa, tea leaves and their beverages, snacks (Coke, Schwebs, Bitter Lemon etc.) (Abulude, 2004).

In United States of America (USA), alcohol is widely consumed though alcohol beverages such as table wines, beers, desert, cordials, liquors, whisky and brandy (Lieber, 2000; Schafer *et al.*, 2001). In Nigeria, alcohol and kolanuts are not only popular in community functions but irregularly consumed. This consumption of alcohol-kolanut has aroused considerable medical interest and could be associated with social behavioral vices. Alcohol and kolanut constituents are believed to have opposing effects on the brain (Osim and Udia, 1993; Adeyeye and Ayejuyo, 1994; Obochi, 2006). Brain function involves subtle chemical and electrical processes, which can easily be altered and modified with the use of psychoactive constituents. These modifications may have metabolic effects on the liver function enzymes-the liver being one of the most important organs in drug metabolism. The activities of two

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amino transferases, AST and ALT are readily used in clinical medicine and toxicology (Allan and James, 1995; Lieber, 1997). There is a possibility of alcohol-kola nuts interaction that brings about changes in the release of these enzymes into the plasma. Elevated levels of AST are occasionally recorded in patients with hepatic disease called intrahepatic cholestasis (Patel *et al.*, 1998; Sokol and Treem, 2001). ALT is relatively higher in the liver than the heart and so it is implicated in hepatic disease (Israel and John, 1994). Determination of AST and ALT and or both, is a useful index of hepatic inflammation as a result of consumption of alcohol kolanuts constituents. The result of this study provides information on the effect of psycho-active constituents of alcohol-kolanut on liver AST and ALT in Albino Wister rats.

MATERIALS AND METHODS

Animal Treatment

Sixteen randomly selected albino rats were obtained from Dr. Utu-Baku's animal house in July, 2006 at Cross River State University of Technology Calabar, Nigeria. The rats weighing between 200-300 g were divided into 4 groups of 4 rats per group and conveyed to the Biochemistry Laboratory.

Group 1 was the control and was fed with normal livestock feed and water *ad libitum*. Group 2 received ground kolanut mixed with feed in the ratio 1:4. Group 3 were fed with normal livestock feed but received alcohol (2% v/v) in their drinking water. Group 4 received a mixture of ground kolanut and fed (1:4) ratio as well as alcohol (20% v/v) in their drinking water. All animals in each group receiving the differed regimens were monitored for 21 days during which their body weights, mortality and food consumed were taken and behavioral tendencies noted.

Materials

All chemicals and solvents used were of reagent grade. The kits for the determination of total protein were obtained from (Linear Chemicals S.L. Toaquin Costa-Barcelona Spain) while those for AST and Alt from RANDOX Laboratories Ltd. Crumlin, UK, sodium hydroxide (4N/Voa/x) and phosphate buffer were supplied by the Departmental Chemical stores.

Homogenization/Collection of Samples

Liver homogenates (WH), postmitochondrial supernatant (pms) and cytosol fractions were obtained by centrifugation (model ALC4217MKII) and made up to 10 mL (phosphate buffer) mark for spectrophotometric measurements of the protein content and enzymes. Total proteins were determined by Biuret method: cepic sulphate 6 mmol L⁻¹ potassium tentarate 2 mmol L⁻¹, potassium iodide 6 mmol L⁻¹ and NaOH 0.75 mol L⁻¹. Estimation of AST and ALT was by the method of Reitman and Frankel (Reitman and Frankel, 1957) using the sample homogenates (WH) and fractions (pms and cytosol).

Measurement of Enzyme Activities AST (GOT) and ALT (GPT) in WH, PMS and Cytosol

Total protein was determined by Biuret reagent with standard Bovine albumin (7 g day⁻¹<); while AST and ALT were estimated using kits (Randox Laboratories Ltd. Crumlin UK). Samples (0.1 pms) used were those obtained from homogenization (WH, pms and cytosol)-mixed, incubated (30 min at 37°C). Enzyme activities (μL⁻¹) of AST/GOT and ALT/GPT were read for absorbance against reagent blank from the table. The data obtained were analyzed statistically using student t-test at 95% (p≤0.05) level where test groups were compared with the control for significant differences.

Data Analysis

The results are presented as the mean standard values of three replicates. Students t-test was used to compare results of each of the test groups with control group at 95% probability level.

RESULTS

The results of treatment of both kolanut and alcohol on the animal specie indicate that there was no significant finding as there were any differences in the results as shown in Table 1. Liver weights were relatively stable, possibly due to the adaptive nature of the liver. Significantly, the value of the effect on food intake (Table 2) shows that 20% alcohol alone was greater than the kolanut consumption for the same period. Albino rats are nibblers and naturally consume their foods in about ten small meals per day. Alcohol may have stimulated the increase of food intake in his group as it was 194.29 ± 24.33 optimal. In Table 3, the effect of kolanut alcohol consumption on the activities of AST and ALT in WH and fractions is illustrated. Significant difference is observed of the two enzymes (AST and ALT) in the WH. AST values are greater than ALT. Low levels of ALT indicate liver sensitivity to the consumption of the constituents of the diets. This is reflected in the pms and cytosolic fractions as well.

The effect of treatment on total protein concentration is shown in Table 4. The result shows that there is a decrease down the group as compared to the control. This trend is marked in the WH and cytosol.

Table 1: Effect of treatment on liver weights of albino rats in treated groups

Groups	Liver weight (g)	Relative liver WH (%)
1	7.45 ± 2.81	3.32 ± 1.60
2	7.37 ± 3.08	3.47 ± 1.08
3	7.61 ± 1.79	3.19 ± 1.31
4	7.74 ± 2.48	3.14 ± 1.97

Table 2: Effect of treatment of constituents on food intakes in Wister albino rats

Groups	Average food intake/21 day
1	147.86 ± 25.99
2	144.22 ± 37.87
3	$194.29 \pm 24.33^*$
4	$135.71 \pm 45.94^*$

*Significantly different from control group; $p \leq 0.05$

Table 3: Effect of alcohol-kolanut consumption on enzyme activities (IU L⁻¹) of AST and ALT in Liver whole Homogenate (WH) and Post Mitochondrial Supernatant (pms) and Cytosolic fractions

Enzyme	Groups	WH	Pms	Cytosol
AST	1	65.75 ± 9.88	18.60 ± 1.10	41.25 ± 2.95
	2	65.50 ± 3.10	$25.38 \pm 1.87^*$	40.12 ± 2.30
	3	60.63 ± 2.90	20.25 ± 1.10	39.38 ± 3.20
	4	$61.00 \pm 1.30^*$	21.65 ± 2.50	$39.35 \pm 4.00^*$
ALT	1	19.38 ± 3.50	15.13 ± 2.00	4.00 ± 0.20
	2	$21.50 \pm 2.20^*$	$10.00 \pm 1.00^*$	$11.50 \pm 0.50^*$
	3	18.50 ± 1.50	14.23 ± 1.20	4.30 ± 0.60
	4	19.60 ± 2.40	$7.00 \pm 1.00^*$	$12.60 \pm 0.40^*$

*Significantly different from the control group $p \leq 0.05$, Group 1 = Control (Normal livestock feed/tap water), Group 2 = Livestock feed/kolanut (4:1), Group 3 = Livestock feed/alcohol (20%v/v), Group 4 = Livestock/kolanut (4:1)/alcohol (20%v/v), All data are means ($\bar{X}$) of these replicates expressed as $\bar{X} \pm \text{SEM}$ $n = 4$, In life observations, show that no deaths occurred as result of alcohol kolanut or the combination of alcohol and kolanut at the end of the 21 day treatment

Table 4: Effect of treatment on total protein concentration (mg mL⁻¹) in whole homogenates (WH) and fractions (pms, Cytosol)

Groups	WH (mg mL ⁻¹)	Pms (mg mL ⁻¹)	Cytosol (mg mL ⁻¹)
1	8.80 ± 2.48	3.24 ± 0.30	5.49 ± 0.39
2	6.78 ± 2.08	3.82 ± 1.08	$3.67 \pm 1.25^*$
3	7.54 ± 1.90	2.50 ± 1.70	$4.18 \pm 1.00^*$
4	$6.08 \pm 2.14^*$	4.16 ± 1.30	$3.45 \pm 0.90^*$

*Significantly different from control group $p \leq 0.05$

The food intakes showed a significant decrease ($p \leq 0.05$) when test groups were compared with the control; with the lowest occurring in the alcohol/kolanut combination (Table 1, 2). However, there was a significant increase in food intake in the group treated with alcohol alone. On the whole, there were no significant differences in the liver weight and their relative values as compared to the control. The average body weights of each group before and after treatment (21 day) indicated significant difference (not shown) ($p \leq 0.05$).

The whole homogenate's (WH) AST values showed a significant increase ($p \leq 0.05$) when compared with the ALT and the highest value was found in the (group 4) treated with livestock feed/kolanut (4:1) alcohol (20% v/v). The pms enzyme activity of AST are equally higher than activity of the ALT but not significant ($p \geq 0.05$) when compared to their control.

The WH total protein concentration (mg mL^{-1}) in the treated group with kolanut-alcohol combination did not show any significant difference when compared with the control group. However, the pms was significant ($p \leq 0.05$) indicating that kolanut-alcohol combination had much effect on the total protein concentration.

DISCUSSION

Speculative inquiry into the effect of random consumption of kolanut-alcoholic beverages is aimed at determining their interactive significance in affecting behavior and liver function enzyme activities in Wister Albino rats. We have shown that alcohol-kolanut constituents administered to Wister Albino rats in a 21 day interval did not result to any death. The treated groups appeared healthy through out the experimental period. However, animals administered alcohol alone and alcohol-kolanut combined were always excited, moving randomly and scattering more of their feeds. The food intakes were significantly increased in alcohol treated group while the groups treated with kolanut alcohol combination showed significant decrease in food intakes. It can be deduced that there was increased appetite in the alcohol treated group. Detection of abnormality in physical behavior, accompanying the ingestion of substances containing caffeine has been reported as physical signs of toxicity (Bosque *et al.*, 1995). Present findings seem to agree with this report that physical toxicity may be established in animal species by close monitoring. Alcohol itself is a central nervous system depressant/sedative while ingredients of kolanuts (theobromine and theophylline) are alkaloids which have been found to have stimulatory effects on the CNS (Palmer, 1991; Eteng *et al.*, 1997; Bladder and Strable, 1998; Gibson and Berger, 2004). The biochemical assays here, evaluates the mechanism of the concomitant effects of alcohol-kolanut constituents on two important liver function enzymes (AST, ALT). The psychotropic effects of alcohol-kolanut interaction can be explained on the basis of the alterations of the levels of these enzymes. Determination of AST, ALT or both is useful in early recognition of toxic hepatitis and is, therefore helpful in studying patients exposed to environmental toxicants (Israel and John, 1994). A high AST/ALT ratio indicates pathology involving the heart, since low levels of ALT among other tissues is found in the heart. A low AST/ALT ratio on the other hand points to the liver since high concentrations of ALT tend to occur in the liver (Utu-Baku, 2000). In this study, the cytosolic levels of ALT in the group of rats treated with alcohol-kolanut regimen were low compared to the AST giving a high AST/ALT ratio. It must be noted that clinical results in humans are quite different from those gotten from animal species like rats and can not be interpreted along these lines.

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