

Biochemical Effects of Energy Drinks Alone or in Combination with Alcohol in Normal Albino Rats

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ABSTRACT

Purpose: To determine the biochemical effects of energy drink alone or in combination with alcohol in normal albino rats.

Methods: Twenty male albino rats weighing 160-180g were assigned into groups A-E of four rats per group. Group A and B rats were given low and high **doses** of ED, respectively, groups C and D were administered low and high **doses** of EDmA, respectively while group E rats were given distilled water and served as control. The treatment lasted for 30 days after which the animals were killed and their blood collected for laboratory analyses using standard methods.

Results: There were no significant differences in body weight, packed cell volume and haemoglobin concentration with either administration of ED or EDmA in comparison to the control. Energy drink alone or EDmA has significant effects on total white blood cell count, plasma potassium, calcium, renal functions, liver enzymes and plasma triglycerides, with EDmA having more effects than ED alone, except for body weight where the energy drink alone has higher effect.

Conclusion: Consumption of energy drink alone or in combination with alcohol is associated with significant alterations in some biochemical parameters. Caution should be exercised while consuming either of them. Public health education is urgently needed to correct the wrong impression already formed by the unsuspecting consumers, especially the youths.

Introduction

The consumption of energy drinks containing large amount of caffeine, taurine and carbohydrates with guarana, ginseng, B-complex vitamins is on the increase,¹⁻³ especially among the youths. It is even more worrisome with the trend permeating the adult and the elderly populations. It is estimated that energy drink consumption among the adolescent and middle-aged population between 2001-2008 ranged from 24-56%.³ A survey of energy drinks consumption among students-athletes in Ghana revealed that 62.2% consumed at least one can of energy drink in a week of which 53.6% did so to replenish lost energy after training and competition.⁴ Other reasons given for consumption of energy drinks included to provide energy and fluid to the body, to improve performance and to reduce fatigue. Attila and Cakir⁵ in a cross-sectional study of college students in Ankara, Turkey showed that frequency of energy drinks consumption was higher among art students and those who were involved in sports, those who did not have breakfast on regular basis, students who ever smoked cigarettes, drank alcohol in comparison to others. In that study, reasons given for consumption of energy drinks

included obtaining energy from it, staying awake, boosting performance while doing sports and mixing with alcohol beverages, with most student unable to correctly define the ingredients of energy drinks, their potential hazardous effects or distinguish between energy drink and sport drink.⁵ In recent times, consumption of energy drinks with alcohol is perceived as a way of ameliorating the toxic effects of alcohol. It has been reported that 20-40% of young people consume energy drink with alcohol while partying.² Although reports suggest that consumption of energy drinks with alcohol reduce awareness of alcohol intoxication and increased alcohol consumption, there are no reliable scientific evidence to support these views.⁶

Reports on the safety of energy drinks consumption have been inconsistent. For instance, while Sionaldo *et al.*⁷ reported no difference in the physiological and biochemical parameters of volunteers consuming energy drinks alone or in combination with alcohol, Ebuehi and colleagues⁸ demonstrated that rats administered energy drinks had higher total protein, triglyceride, HDL-, LDL-cholesterol and glucose, but

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lower ALT, AST, creatinine, uric acid and albumin, without histopathological abnormalities of the brain, heart and liver. It has been suggested that combining energy drinks with alcohol as practised by young people exacerbates safety concerns.³ It has been reported that long-term exposure to the various components of energy drinks may result in significant alterations in the cardiovascular system.⁹ Again, cases of caffeine-associated deaths and seizures have been identified.^{10,11} Also, Usman and Jawaid¹ reported a case of hypertension in a young boy due to energy drink consumption. While research is yet to be conclusive on the safety or otherwise of energy drink consumption, the International Society of Sport Nutrition,¹² and the Committee on Nutrition and the Council on Sports Medicine and Fitness¹³ recently expressed concerns on the safety and efficacy of the use of energy drinks. The present study is therefore aimed at investigating the biochemical effects of energy drink alone or in combination with alcohol as widely practiced today in normal albino rats.

Materials and Methods

Animals and sample collection

Twenty male albino rats weighing 160-180 g used for this study were purchased from animal house of the Department of Pharmacy, University of Nigeria Nsukka. The rats were randomly assigned into five groups (A-E) of four rats per group in a cage and housed in the animal house of Biochemistry Department of Ebonyi State University, Abakaliki. The animals were allowed to acclimatise for 7 days under standard environmental conditions and maintained on a regular livestock feed (Pfizer Plc, Lagos, Nigeria) and water *ad libitum*. Thereafter rats in groups A (LED) were administered low dose of energy drink only (3.75ml/Kg Bullet[®]), group B (HED) rats were given high dose of energy drinks only (7.5ml/Kg Bullet[®]) while rats in groups C (LEDmA) were co-administered low dose of energy drink plus alcohol (3.75ml/Kg energy Bullet[®] + 1.0g/Kg alcohol) and rats in group D (HEDmA) were given high dose of energy drink plus alcohol (7.5 ml/Kg Bullet[®] + 2.0 g/Kg alcohol). Group E rats served as controls and were given only water. All the rats received human care in accordance with the National Institute of Health guidelines for the care and use of laboratory animals.¹⁴ The experiment lasted for 30 days after which the animals were fasted for 12 hours before they were sacrificed and their blood collected for haematological and biochemical analyses. Blood for haematological parameters and lipid profile

was collected in EDTA bottle while blood for other biochemical parameters was collected in lithium heparin bottle. Immediately the haematological parameters were completed, the blood was spun in a laboratory centrifuge at 2000 g for five minutes and plasma separated and stored at -4°C prior to analysis. All the analyses were done within 24 hours of sample collection.

Laboratory analyses

Haematological parameters; packed cell volume (PCV), haemoglobin concentration (HBC) and total white blood cell counts (TWBC) were done as described by Dacie and Lewis.¹⁵ Plasma calcium (Ca) was determined by atomic absorption spectrophotometer while sodium (Na⁺) and potassium (K⁺) were determined using flame photometers. Plasma albumin was determined by colorimetric bromocresol green methods as described previously,¹⁶ while uric acid was determined by uricase method as described by Fossati *et al.*¹⁷ Plasma urea was determined by the method described by Jung *et al.*¹⁸ and creatinine estimation was done by method originally described by Benedict and Behie¹⁹ and reevaluated by Stevens *et al.*²⁰ Total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay as described previously^{21,22} and HDL-cholesterol was determined enzymatically after precipitation of other lipoprotein as described by Warnic *et al.*²³ while LDLcholesterol was calculated using Friedewald equation.²⁴ Plasma Alanine transaminase (ALT), Aspartate transminases (AST), alkaline phosphatase (ALP) and bilirubin were determined using test kits (Randox Laboratories, UK) in accordance with manufacturer's instructions.

Data analysis

The data generated were analysed with Statistical Package for Social Sciences (SPSS) version 16. Results were expressed as mean \pm standard deviation with differences between means determined by one way analysis of variance (one-way ANOVA). Statistical significance was achieved at p-values < 0.05.

Results

Table 1 reveals that energy drink alone had more effect on body weight than in combination with alcohol, with 2.0% and 2.9% change in body weight observed for low and high dose of energy drink only, respectively as against 1.8% and 1.7% for high and low dose of energy drink plus alcohol, respectively.

Table 1. Effects of energy drink alone or in combination with alcohol on body weight of normal albino rats

Body weight (Kg)	Group A	Group B	Group C	Group D	Control
Initial	171.6 \pm 1.3	168.5 \pm 0.9	174.1 \pm 1.5	173.2 \pm 1	172.9 \pm 1.1
Final	175.1 \pm 0.7	173.4 \pm 1.2	177.3 \pm 0.6	176.1 \pm 1.7	175.1. \pm 1.3
Change (%)	3.5 (2.0)	4.9 (2.9)	3.2 (1.8)	2.9 (1.7)	2.2 (1.3)

Table 2 shows that consumption of energy drinks either alone or in combination with alcohol has no effect on packed cell volume (PCV) and haemoglobin concentration (HBC) as comparable values were observed in all the groups. However, significantly lower (4.3 ± 0.3 and $4.9 \pm 0.3 \times 10^9/L$) and higher (6.2 ± 0.3 and $6.7 \pm 0.2 \times 10^9/L$) total white blood cell counts

(TWBC) were recorded in rats administered energy drink alone and energy drink and alcohol, respectively in comparison to normal rats not administered energy drink or alcohol ($5.4 \pm 1.4 \times 10^9/L$). Neither the energy drink alone nor energy drink plus alcohol at higher doses has significant effect on the TWBC (Table 2).

Table 2. Effects of energy drink alone or in combination with alcohol on haematological parameters of normal albino rats

Parameters	Group A	Group B	Group C	Group D	Control
PCV (%)	46.0 ± 7.4	44.7 ± 5.5	51.3 ± 9.8	51.5 ± 3.5	51.0 ± 3.7
HBC (g/dl)	15.2 ± 2.4	14.8 ± 1.8	16.8 ± 3.1	16.9 ± 1.3	16.9 ± 1.2
TWBC ($\times 10^9/L$)	4.3 ± 0.3^a	4.9 ± 0.3^a	6.2 ± 0.3^b	6.7 ± 0.2^b	5.4 ± 1.4^c

While consumption of energy drinks has no effect on plasma sodium, significantly lower and higher plasma potassium were observed with consumption of energy drinks alone and energy drinks plus alcohol, respectively, although no significant difference was observed with either consumption of energy drinks alone or energy drinks plus alcohol at higher doses (Table 3). On the other hand, plasma calcium was comparable among rats administered energy drinks

alone and those administered energy drinks and alcohol, but values were significantly ($p = 0.04$) higher than those observed in rats not administered energy drinks alone or energy drinks plus alcohol. Plasma urea, uric acid and creatinine were significantly ($p < 0.05$) affected only at higher doses of energy drinks and alcohol combination, although plasma urea and creatinine were generally higher in the experimental rats than in the controls (Table 3).

Table 3. Effects of energy drink alone or in combination with alcohol on plasma electrolytes, creatinine and urea of normal albino rats

Parameters	Group A	Group B	Group C	Group D	Control
K^+ (mmol/l)	3.52 ± 0.07^a	3.57 ± 0.07^a	3.82 ± 0.21^b	4.05 ± 0.09^c	3.79 ± 0.24^d
Na^+ (mmol/l)	100.4 ± 3.4	98.4 ± 10.7	102.6 ± 2.8	105.9 ± 1.7	99.7 ± 1.7
Ca^{++} (μ mol/l)	2.06 ± 0.07^a	2.05 ± 0.09^a	1.92 ± 0.15^a	2.07 ± 0.25^a	1.77 ± 0.27^b
Urea (mmol/l)	3.32 ± 0.58^a	3.83 ± 0.31^a	3.38 ± 0.71^a	4.60 ± 0.28^b	3.05 ± 0.58^c
Uric acid (μ mol/l)	119.8 ± 5.4^a	129.3 ± 14.2^a	140.0 ± 11.3^a	163.5 ± 10.6^b	140.5 ± 22.0^a
Creatinine (μ mol/l)	40.6 ± 7.7^a	38.5 ± 4.2^a	44.6 ± 7.8^a	45.6 ± 15.2^b	29.2 ± 5.4^c

From Table 4, while comparable plasma albumin was observed in experimental rats and controls, significantly ($p < 0.05$) higher ALT was recorded in experimental rats in general in comparison to the

controls, with significantly higher levels of ALP, AST and total bilirubin observed only in experimental rats co-administered higher doses of energy drinks and alcohol.

Table 4. Effects of energy drink alone or in combination with alcohol on liver function parameters of normal albino rats

Parameters	Group A	Group B	Group C	Group D	Control
Albumin (g/dl)	3.2 ± 0.4	3.1 ± 0.4	3.2 ± 0.2	3.3 ± 0.7	3.4 ± 0.5
ALT (U/L)	11.8 ± 1.7^a	14.7 ± 1.5^a	12.0 ± 3.5^a	13.0 ± 1.4^a	9.5 ± 1.3^b
ALP (U/L)	26.3 ± 5.1^a	28.7 ± 4.2^b	38.0 ± 7.7^c	43.5 ± 6.4^c	29.8 ± 2.8^d
AST (U/L)	12.3 ± 2.2^a	16.1 ± 1.0^b	14.8 ± 3.0^a	16.0 ± 1.4^b	14.5 ± 2.4^a
Total Bilirubin (μ mol/l)	11.4 ± 0.9^a	14.2 ± 3.8^a	13.6 ± 1.6^a	16.10 ± 3.0^b	12.8 ± 1.2^a

Table 5 shows the effects of energy drink alone or in combination with alcohol on lipid profile of normal

rats. Although comparable levels of plasma total cholesterol, HDL-cholesterol and LDL-cholesterol

were observed among the groups, significantly ($p < 0.05$) higher plasma triglyceride was observed in rats co-administered energy drink plus alcohol in

comparison to those administered energy drink alone and the control.

Table 5. Effects of energy drink alone or in combination with alcohol on plasma lipids of normal albino rats

Parameters	Group A	Group B	Group C	Group D	Control
Total Cholesterol (mmol/l)	3.8 ± 0.5	4.2 ± 0.4	4.0 ± 0.6	4.0 ± 0.3	4.2 ± 0.2
HDL-Cholesterol (mmol/l)	0.82 ± 0.09	0.81 ± 0.26	0.70 ± 0.14	0.62 ± 0.28	0.79 ± 0.12
LDL-Cholesterol (mmol/l)	1.5 ± 0.3	1.8 ± 0.3	1.5 ± 0.3	1.7 ± 0.1	1.4 ± 0.1
Triglyceride (mmol/l)	1.3 ± 0.2 ^a	1.4 ± 0.2 ^a	1.7 ± 0.4 ^b	1.9 ± 0.3 ^b	1.4 ± 0.1 ^a

Discussion

The present study has shown that energy drink alone or in combination with alcohol has variable effects on total white blood cell count, plasma potassium, calcium, renal functions, liver enzymes and plasma triglycerides, with combination of energy drink and alcohol having more effects than energy drink alone, except for body weight where the energy drink alone has higher effects.

Higher increase in body weight with consumption of energy drink alone than in energy drink plus alcohol observed in the present study is understandable as the excess energy consumed but not utilised are stored and impact on body weight. Although study on the effect of energy drink alone or in combination with alcohol on body weight was not encountered, alcohol consumption has been associated with increase burning of calorie. Also, evidences relating energy drink consumption with weight loss are confliction but data suggest that combination of energy drink with exercise enhances body fat reduction.³

The significantly higher total white blood cell count observed in rats co-administered energy drink and alcohol observed in this study may reflect the effect of alcohol on the immune system. For instance chronic consumption of alcohol has been associated with malnutrition, particularly micronutrient deficiencies. Micronutrient deficiencies, especially deficiencies of zinc, are known to promote immunodeficiency, which will ultimately encourage infections with attendant mobilisation of white blood cells to fight the infections. According to Ebuehi and colleagues,⁸ consumption of energy drink was associated with higher total protein, triglyceride, HDL and LDL but lower ALT, AST, creatinine, uric acid and albumin, which is in contrast with the present findings of significantly higher urea, uric acid, creatinine and liver enzymes and total bilirubin, especially in rats co-administered high dose of energy drink and alcohol. Although the reason for this disparity is not obvious, it may be partly attributed to toxic effect of alcohol. Consumption of energy drink with alcohol has been associated with increased risk of serious injury, sexual assault, drink-driving alcohol-related health consequences.² Furthermore, elevated plasma urea, uric acid and creatinine in rats

administered energy drinks alone or energy drink plus alcohol in comparison to control suggest renal involvement. Both urea and creatinine are products of protein metabolism, which are accumulated in the blood when the kidneys are affected.

The present findings may have public health implications for those that are forming the habits of combining energy drink with alcohol with erroneous belief that it ameliorates the effects of alcohol. The elevated liver enzymes observed in rats administered energy drink alone and energy drink plus alcohol suggests adverse effects of the drinks on the liver. Regrettably, liver histopathology was not examined to confirm liver injury. These findings also reaffirm the recent stand of The International Society of Sports Nutrition on energy drinks that diabetics and individuals with pre-existing cardiovascular, metabolic, hepatorenal, and neurologic disease, who are taking medications that, may be affected by high glycaemic load foods, caffeine, and/or other stimulants should avoid use of energy drink (ED) and/or energy shots (ES) unless approved by their physician.¹² Although our subjects were normal rats that were maintained on normal rat feed and water, it may be speculated that the variations in biochemical parameters attributed to either consumption of energy drink alone or in combination with alcohol may be exacerbated in diabetic subjects. Previously, combining energy drink with alcohol has been suggested to exacerbate safety concerns.³ Significantly elevated plasma potassium in rats administered either energy drink alone or energy drink plus alcohol observed in the present study has important health implication. Potassium is very important to the heart as either low or high plasma potassium levels may lead to cardiac abnormalities. It has previously been reported that long-term exposure to the various components of energy beverages may result in significant alterations in the cardiovascular system.⁹ Also consumption of energy drinks containing high and unregulated amount of caffeine has been associated with serious adverse effects, such as seizures, diabetes mellitus, cardiac abnormalities or mood and behavioural disorders, particularly in children, adolescents and young adults and those who take certain medications.²⁵ Hence it may be inferred that the

health abnormalities previously associated with consumption of energy drink may have been mediated through the alteration in plasma potassium. The cardiac abnormalities may also be partly attributed to disorder in lipid metabolism as evidenced by significantly elevated plasma triglyceride in rats administered energy drink plus alcohol observed in the present study. Dyslipidaemia has been associated with cardiac abnormalities.²⁶ Usman and Jawaid¹ had earlier reported a case of hypertension in a young boy consuming energy drink. It is yet to be ascertained whether the elevated triglyceride observed in the present study is due to the effects of alcohol or energy drink or both.

Conclusion

It may be concluded that consumption of energy drink alone or in combination with alcohol is associated with significant alterations in some biochemical parameters. In view of paucity of data on the safety and efficacy of energy drink alone or energy drink plus alcohol, either should be consumed with caution.

Conflict of Interest

The authors declare that they have no conflict of interest.

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