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Research Article

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Consumption of caffeinated energy drink induces alterations in lipid profile and hepatic aminotransferases in experimental rats

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ABSTRACT

Public health concerns have been associated with the widespread consumption of energy drinks. However, there is a dearth of scientific data on the biochemical effects of these energy beverages. This study aimed to investigate the effect of caffeinated energy drinks commonly consumed in Nigeria on plasma lipids, lipid peroxidation marker and liver function indices in albino rats. Male Wistar rats were administered orally caffeinated energy drinks (1mL/100g body weight) for 28 days. At the end of the experimental period, plasma lipid profile, lipid peroxidation marker (malondialdehyde) and liver indices were estimated in control and experimental groups using standard methods. P<0.05 was considered for statistical significance. Treatment with energy drinks (EDs) demonstrated decreases in total cholesterol, LDL-C, while TG increased as comparable to control. However, concentrations of HDL-C was significantly reduced, while lipid peroxidation marker (malondialdehyde) was significantly increased (p<0.05) by Tiger ED. Although energy drink administration altered hepatic aminotransferases insignificantly (p>0.05) at 1mL/100g body weight, Wild Dragon ED increased the activity of aspartate aminotransferase significantly as compared to control. Alterations in lipid profile, hepatic status and the observed lipid peroxidation by caffeinated ED consumption may have important public health implications. We therefore suggest further investigations in this direction.

Key words: Energy drink, Malondialdehyde, Lipid peroxidation, Aminotransferases, Caffeine.

INTRODUCTION

Worldwide consumption of energy drinks (EDs) is exponentially increasing due to their stimulant effect on the central nervous system and body, and the purpose of enhancing both cognitive and physical performances [1]. Consumption of sports drinks, a mixture of carbohydrates and electrolytes, is common among the athletes for ergogenic effects, enhanced twitch strength for cardiac muscle and muscle endurance [2, 3]. EDs are non-alcoholic beverages that contain caffeine in combination with other ingredients of herbal extracts such as guarana, ginseng, and ginkgo biloba, B vitamins, amino acids such as taurine, derivatives of amino acid as carnitine, and sugar derivatives, including glucuronolactone and ribose [4]. Although they seem like a new trend, these drinks have been available to the general public for some time. For instance, the first ED was launched in Japan in 1960 [5], introduced to Europe (Austria) in 1987 before quickly expanding throughout the rest of Europe, and to the United States and Nigeria in 1997 [5, 6]. Traditionally, intake of EDs is associated with the athletes for extra energy,

increased physical and psychological performances. Currently, ED consumption is common in the general population, particularly among the adolescents or young adults and even senior citizens [7]. Manufacturers of these products promote their consumption, targeting the young population to offer them benefits for better energy, mental alertness, improved emotional state, stimulatory effects and weight loss [7, 8]. The full impact of the rise in popularity of energy drinks has not yet been quantified, but the aggressive marketing of energy drinks with caffeine content up to 505 mg/can or /bottle combined with limited and varied regulations have created an environment where energy drinks could pose a significant threat to public health [5, 9].

The major ingredients of energy drinks are caffeine, taurine and guarana and are usually present at high concentrations [1]. The high caffeine content in EDs has generated public health concerns and challenging consumer safety. Recently, the International Society of Sports Nutrition [10] and the Committee on Nutrition and the Council on Sports Medicine and Fitness [11] expressed concerns on the safety and efficacy of ED consumption. Some studies suggest that ED consumption may adversely affect cardiovascular health and hematopoietic system [8, 12, 13, 14], with various side effects and death [14, 15].

However, the biochemical effects of combination of ingredients in caffeinated EDs for acute and chronic toxicity are not well known. Limited studies have assessed effects of ED consumption on biochemical indices associated with the heart, kidney, liver, haematopoietic system, and neurological seizures [8, 16-24]. Ugwuja et al [25] reported alterations in biochemical parameters of rats administered energy drink alone or in combination with alcohol. The inconsistent results make it difficult to draw a unifying conclusion on the biochemical effects of ED consumption on public health. This study therefore aimed at assessing the effects of three popular energy drinks commonly consumed in Nigeria (Power Horse, Wild Dragon and Tiger) on plasma lipids profile, lipid peroxidation marker and liver function aminotransferases in rats.

EXPERIMENTAL SECTION

Animals

Sixteen adult male Wistar rats weighing 140±20g were used for this study. The animals were allowed to acclimatize for 7 days and were maintained on standard commercial rodent feed with water *ad libitum*. The rats were kept in a standard animal house in metallic cages with a 12 hour light/dark cycle. Throughout the experiment, room temperature was maintained at 25 ± 2 ^{o}C. All the rats received humane care in accordance with the National Institute of Health guidelines for the care and use of laboratory animals [26].

Experimental Design

The EDs were purchased from the local commercial shops in Abakaliki, Ebonyi State, Nigeria. The major constituents of these EDs were caffeine, guarana, taurine, ginseng, B vitamins and carbohydrates. The animals were divided into 4 groups of 4 animals each. Group 1 animals were kept on normal rodent diet and water and served as control, while animals in Group 2, 3 and 4 were orally administered a single daily dose (1mL/100g of body weight) of Power Horse, Wild Dragon and Tiger, respectively, for 28 consecutive days. On the 29th day, after an overnight fasting period, the rats were sacrificed by cervical dislocation and blood samples collected by cardiac puncture into heparinized tubes. The blood was centrifuged at 3000 rpm for 15 minutes at the Departmental Laboratory of Physiology, Ebonyi State University, Nigeria. The plasma obtained was stored at -4 ^{o}C for analyses of lipid profile, MDA and liver function indices. Liver tissues were carefully excised and preserved using 10% buffered formalin.

Biochemical Analyses

Determination of Plasma Lipids

Plasma total cholesterol (TC) and triglyceride (TG) concentrations were determined by enzymatic colorimetric assay as described by Siedel et al [27] and Fossati and Precipe [28] HDL-cholesterol (HDL-C) was determined enzymatically after precipitation of other lipoproteins as described by Warnic et al [29] using commercial kits from Randox Laboratories Ltd, Crumlin, UK. LDL-cholesterol (LDL-C) was calculated using Friedewald formular [30].

Liver Function Analyses

Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to the method of Reitman and Frankel [31] using test kits (Randox Laboratories, UK) in accordance with the manufacturer's instructions. Total protein (TP) was determined by the Biuret method of Weichselbaum [32] while

plasma albumin (ALB) was determined by colorimetric bromocresol green method as described by Doumas et al [33].

Lipid Peroxidation

Thiobarbituric acid reactive substances, measured as malondialdehyde (MDA), were determined in plasma. In the present study, plasma MDA, a product of lipid peroxidation, was determined spectrophotometrically as described by Wallin et al [34]

Histological examination

The liver from rats of different groups for histopathological examinations was collected after sacrifice. These tissue samples were fixed in 10% neutral buffered formalin, dehydrated in graded alcohol and embedded in paraffin. Fine sections obtained were mounted on glass slides and counter-stained with hematoxylin–eosin (H&E) for light microscopic analyses. The slides were examined by a histopathologist who was blinded to the treatment groups after which photographs were taken.

Statistical Analysis

The data were expressed as mean ± SD. Differences between group means were estimated using a one-way ANOVA followed by Tukey statistical test, using SPSS version 20.0 for Windows (SPSS Inc., Chicago IL, USA). Results were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

A common concern that is expressed about caffeinated energy drink consumption is that these products may emerge as energy beverages with clinical or sub-clinical toxic effects on the consumers. The combinatorial influence of ED ingredients is important for public health safety especially for the unsuspecting consumers between the age of 13 and 35 years [17] largely the young adults. To our knowledge, few studies have been carried out to assess possible biochemical effects of ED consumption in animals and humans. In this context, the present study evaluates biochemical effects of EDs in sub-acute exposure in albino rats. In this study, ED administration to grouped rats demonstrated disruption in lipid profile, liver function and formation of peroxidative product. We found that after 28 days, there were alterations in biochemical indices that demonstrated the effect of these products.

Plasma lipids

Table 1 presents the effects of energy drinks on lipid profile measured in control and experimental groups during the study. Energy drink administration demonstrated decreases in TC, HDL-C, and LDL-C, whereas an increase was observed for TG, as compared to control. However, Tiger ED significantly ($p=0.03$) decreased HDL-C in comparison to control. The EDs decreased insignificantly the lipoprotein cholesterol profile, and increase in TG was observed by the same treatment. Lipid profile is important for dyslipidemia and associated with atherosclerosis, diabetes, obesity and other degenerative disorders [35]. HDL-C level has been inversely linked to the risk of cardiovascular diseases by several studies. However, although the effects on TC and LDL-C may not signal adverse health impact of ED consumption, it is noteworthy that we observed a significant decrease in HDL-C induced by Tiger ED (Table 1). In addition, TG, an atherogenic plasma lipid rich in apo C-III [35] increased, although insignificantly in all ED administration.

Table 1: Effects of administration of energy drinks on plasma lipid profile in rats

Experimental group	TC(mmol/L)	HDL-C(mmol/L)	LDL-C(mmol/L)	TG(mmol/L)
Control	4.03 ± 0.37	1.70 ± 0.12	2.02 ± 0.46	1.55 ± 0.93
Power Horse	3.58 ± 0.38	1.48 ± 0.36	1.46 ± 0.52	1.65 ± 0.87
Wild Dragon	3.70 ± 0.62	1.43 ± 0.44	1.79 ± 0.41	2.08 ± 1.02
Tiger	3.53 ± 0.78	1.35 ± 0.30*	1.98 ± 0.16	1.73 ± 0.94

*Values are mean ±SD. Significantly different from control: *p<0.05*

The effect of decrease in HDL-C and increase in TG may be significantly amplified in a chronic consumption of these drinks. At present, limited literature is available on the effect of ED on lipid profile to compare results obtained in this study with. The available literature by Ugwuja et al [25], although inconsistent with our finding for HDL-C, reported that ED may not have important effect on lipid profile after the administration of a different brand of energy drink. However, over the past research, there is a line of evidence on the effect of caffeine, the major

content of an ideal ED, on lipid profile and predisposition to atherogenesis [36]. A number of experimental and human studies have shown significant alterations in lipid parameters induced by caffeine consumption [36-38]. Conclusions of other studies pointed out that it is very likely that most of the observed effects after consumption of EDs are mainly produced by caffeine content [7, 39]

Liver function parameters

The effects of administration of energy drinks on plasma liver function were shown in Table 2. Treatment with Power Horse, Wild Dragon, or Tiger generally caused insignificant decreases ($p>0.05$) in TP, AST and ALT as compared to control. However, AST was significantly increased ($p<0.05$) by Wild Dragon, while the increase observed in ALT by the same energy drink was insignificant ($p>0.05$). ALB concentration was insignificantly reduced in rats administered with Power Horse. Comparatively, the potential of EDs to undermine liver functions are more assessed and reported in scientific literature than for lipid profile. Such skewness in available data may be related to the important role of the liver in caffeine metabolism. Caffeine in EDs is metabolised by the hepatic cytochrome P4501A2 (CYP1A2) enzyme in a series of demethylation reactions to 1-methylxanthine and 1-methyluric acid, the main metabolites of caffeine [40]. AST and ALT are useful plasmatic parameters of hepatic injury that are associated with liver toxicity.

Table 2: Effects of administration of energy drinks on liver function parameters in rats

Experimental group	TP (g/dl)	ALB(g/dl)	AST(IU/L)	ALT(IU/L)
Control	4.13 ± 0.62	2.20 ± 0.77	154.03 ± 31.20	40.73 ± 19.55
Power Horse	3.65 ± 0.30	1.80 ± 0.68	145.45 ± 37.14	35.22 ± 6.05
Wild Dragon	3.70 ± 0.00	2.25 ± 0.66	195.13 ± 21.04*	48.08 ± 11.72
Tiger	3.83 ± 0.55	2.25 ± 0.52	142.95 ± 8.52	33.78 ± 5.79

*Values are mean ± SD. Significantly different from control: *p<0.05*

Here, we observed inconsistent changes in liver function parameters. Power Horse and Tiger insignificantly decreased AST and ALT, whereas Wild Dragon administration increased the activities of the enzymes, particularly significantly in AST (Table 2). This observation is consistent with the previous experimental findings of studies [17, 24, 25] on the effect of ED consumption. Although caffeine is the most active ingredient of EDs, herbal and non-herbal ingredients like yerba mate, ginkgo biloba, B vitamins and L-carnitine associated with prevention of cell damage and antioxidant properties added in variable proportions in brands [9, 41], may be associated with the beneficial effect of Power Horse and Tiger ED on plasma levels of liver enzymes. On the other hand however, Wild Dragon ED demonstrated toxic effect on liver tissue evidenced by significant increase in AST. Energy drink brands with low constituents known for antioxidant potential may have deleterious synergistic effect on hepatic status. Meanwhile, Reissin et al [39] have reported that adverse reactions and toxicity from high-energy drinks stem primarily from caffeine content. In support, studies suggest that caffeine administration significantly increased AST, ALT and creatinine in serum of normal and obese diabetic rats [42, 43]. Decrease in total protein in this study is consistent with the observed decrease in ALT and AST. Our report for total protein and albumin contradicts a study report that ED consumption is associated with increase in total protein and decrease in albumin [24]. It may be prudent to attribute this discrepancy to administration of different brands of ED with variable constituents.

Lipid Peroxidation

The effects of EDs on malondialdehyde (MDA) are summarized in Fig 1. We observed that ED consumption by animals increased concentrations of MDA. Tiger energy drink induced lipid peroxidation such that MDA level significantly increased ($p<0.001$) as compared to control. The observed lipid peroxidation by increased plasma malondialdehyde suggests possible potential of EDs to induce tissue damage, although our data for this marker were comparable to control except Tiger-treated group that demonstrated significant increase in malondialdehyde at $p<0.001$ (Figure 1). To our knowledge, there are no studies that have considered lipid peroxidative potential of ED consumption.

Histological findings

The photomicrographs of rat liver section from control and treatment groups are shown in Fig 2. The histological structure of the liver was observed to be normal in the control group (Fig 2A). Hepatic section of rat treated with the EDs showed congestive and proliferative alterations of hepatic tissues with moderate infiltration of inflammatory cells (Fig 2B-D).

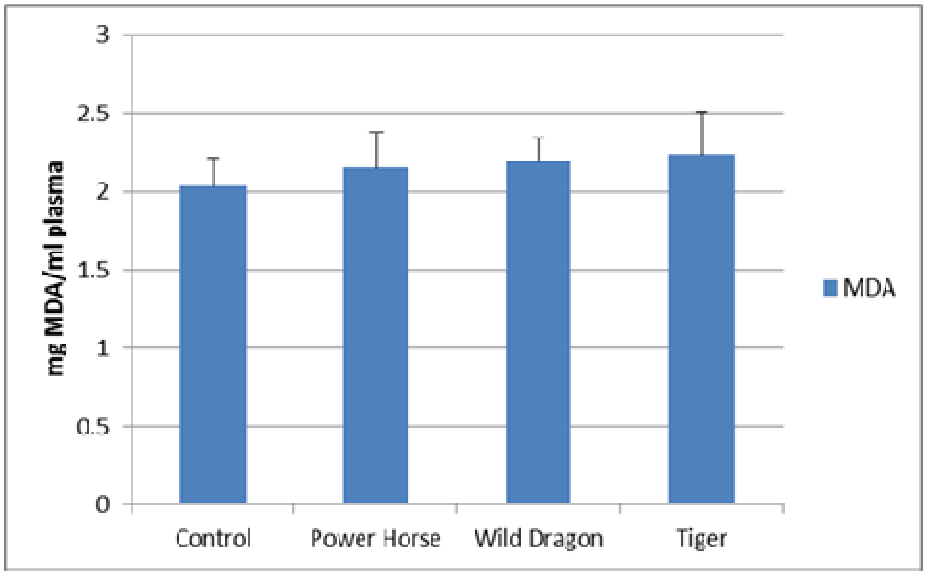

Fig 1: Effects of energy drink consumption on plasma malondialdehyde in albino rats

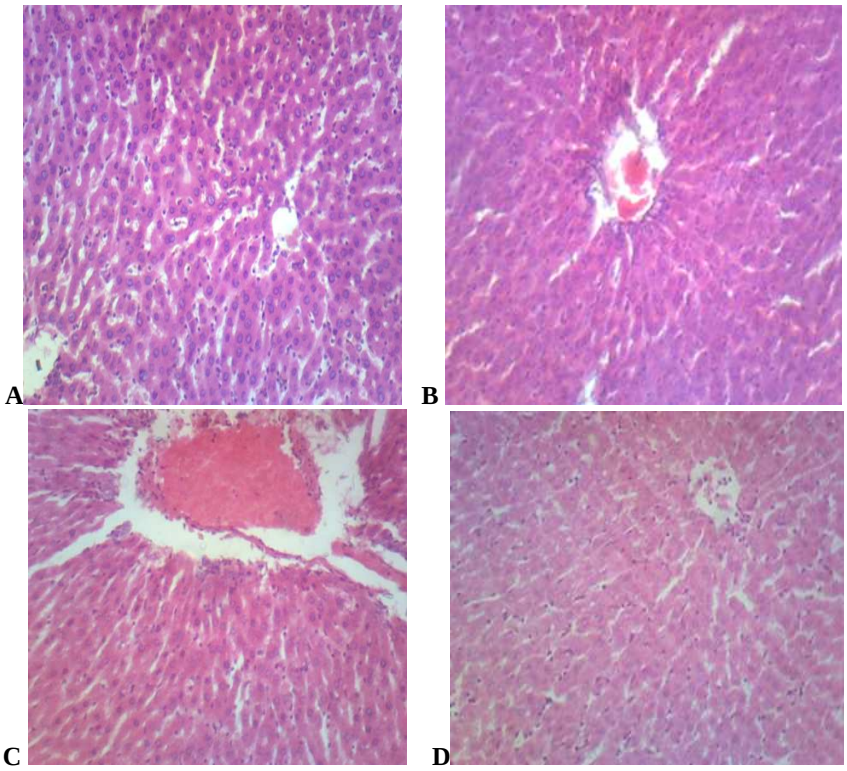

Fig 2: Photomicrograph of hepatic tissue of rats from the four experimental groups (H-E, 150 µm): A-normal liver histology of rat (Control); B -congestion and perfusion of hepatic tissue (Power Horse); C- congestion and increased perfusion of hepatic tissue with proliferation of bile duct (Wild Dragon); D: increased portal activity and fusion of near-by hepatocytes with moderate infiltration of inflammatory cells (Tiger)

However, accumulating evidence suggests a potential antioxidant role for caffeine [1, 2, 44, 45], although the free radical scavenging ability has been associated with physiologically acceptable amount of caffeine in some studies [40, 46], and many studies suggest that caffeine concentrations in EDs are above the acceptable level [9]. However, the beneficial role of caffeine reported in previous studies remains to be reported for the considerably high amount

of caffeine in EDs, and this is important for public health safety. The architectural alterations found in rat liver sections confirm possible effects of these beverages.

CONCLUSION

The present study shows that Tiger and Wild Dragon energy drinks available on the Nigerian markets may deleteriously impact on biochemical parameters with important implications on public health. We hereby highlight the need for further investigations in this direction. Meanwhile, for protection of public health, it is prudent that energy drinks be cautiously and minimally consumed.

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