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***Research Article** (Open Access)*

EFFECT OF ACETAMINOPHEN AND CAFFEINATED ENERGY DRINK ON LIVER ENZYMES ACTIVITIES OF WISTAR ALBINO RATS DURING SUB-CHRONIC ALCOHOL CONSUMPTION

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ABSTRACT
The effect of administering acetaminophen and energy drink on liver enzymes; aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) during sub-chronic alcohol consumption in Wister albino rats was investigated. The forty-two Wistar albino rats used were divided into seven groups of six rats each. Group 1 served as the normal control and received 1ml of bottled water, group two received alcohol (2.5ml/kg body weight of Smirnoff vodka (40% v/v)), group three was given energy drink (5ml/kg body weight of power horse) while group four received paracetamol (28.55mg/kg body weight), group five received same dose of alcohol and energy drink, group six received same dose of alcohol and paracetamol, and group seven received same dose of alcohol, energy drink and paracetamol. The administration was carried out twice daily for 14 days. The results obtained showed that there were significant increases ($p< 0.05$) in the levels of AST and ALT in the groups treated with alcohol (50.25 ± 1.93 and (50.75 ± 0.85) confirming the hepatotoxic effect of alcohol. While the enzymes pattern in the energy drink group was comparable to those of the normal control, paracetamol administration alone caused non-significant increases ($p>0.05$) in the levels of AST, ALT and ALP. Administration of alcohol with energy drink produced a significant decrease ($p<0.05$) in ALT level (µmol/l) and a non-significant reduction in AST (µmol/l) compared to the alcohol group. Alcohol administration with paracetamol caused significant reductions in ALT and AST levels and a non-significant reduction in ALP level (µmol/l) compared to the alcohol group. In conclusion, it is observed that paracetamol administration during sub-chronic alcohol consumption as well as consumption of energy drink, individually or in combination with paracetamol results in improved hepatic outcomes compared to the consumption of alcohol alone.

***Keywords**: Alcohol, Acetaminophen, paracetamol, Energy drink, Caffeine*

INTRODUCTION
An alcoholic beverage is a drink containing ethanol, commonly known as alcohol. They are divided into three general classes: beers, wines, and spirits. They are legally consumed in most countries, and over 100 countries have laws regulating their production, sale, and consumption. The production and consumption of alcohol occurs in most cultures of the world, (Alford *et al.*, 2001), it is often an important part of social events in these cultures. In many cultures, drinking has been known for its significant role in social interaction, mainly because of alcohol's neurological effects Cheul Do *et al.*, 1997). High blood alcohol content is usually considered to be legal drunkenness because it reduces attention and slows reaction speed. Alcohol can be addictive, and the state of addiction to alcohol is known as alcoholism (Alford *et al*., 2001). It has also been found out that, Alcoholic beverages that have lower alcohol content like wines are produced by fermentation of sugar or starch-containing plant material. Beverages of higher alcohol content (spirits) are produced by fermentation followed by distillation. Due to its toxic and depressant effect, people who drink now mix the alcoholic beverage with energy drinks.

Dietary supplements may prevent or relieve some of alcohol's harmful effects. For example, brain damage resulting from a lack of vitamin B_1, which can lead to conditions such as Wernicke–Korsakoff syndrome, can be reversed to some extent. Because vitamin B1 generally can be administered safely, clinicians often recommend that all alcoholics undergoing treatment receive 50 milligrams of thiamine

per day (either by injection if the patients are hospitalized or by mouth). Alcoholics also should receive supplements of vitamins B_2 (riboflavin) and B_6 (pyridoxine) in dosages found in standard multivitamins. Vitamin A, however, can be toxic when combined with alcohol (Lieber, 1991).

Energy drinks are non-alcoholic, often lightly carbonated beverages that are designed to give the consumer a burst of energy by the addition of a number of energy enhancing ingredients, most notably caffeine. They are designed to improve physical attributes, and the mental alertness of the consumer. Unlike regular soft drinks, these drinks have more caffeine, the addition of herbal supplements, and extra vitamins. They stimulate a person's senses to give them more energy, while revitalizing the body. Energy drinks commonly include caffeine, other plant based stimulants (guarana, ephedrine,yerba mate), simple sugars (glucose, fructose), amino acids (taurine, carnitine, creatine), herbs (various forms of ginseng, ginkgo biloba), maltodextrin, inositol, and glucuronolactone (Alford *et al.,* 2001).

Energy drinks on their own contain some amount of caffeine, from high to low contents of caffeine depending on the type of energy drink (Marczinski *et al.,* 2011). The reason for the mixture, being to reduce the intoxicating effect of alcohol. The mixing of energy drinks with alcohol has now led to people drinking more alcohol than they would have taken alcohol alone without the energy drink. (Ligouri *et al.,* 1997).This large intake of alcohol leads to a hangover, and paracetamol is taken to reduce the headache and other problems associated with alcoholic hangover.

MATERIALS AND METHODS

Collection and preparation of materials

Smirnoff vodka (40% v/v) and power horse obtained from Sparkz shop in Calabar were used as alcohol and energy drink respectively. Emzor paracetamol was obtained from Obel pharmacy in Calabar.

Experimental animals

Forty-two wistar albino rats weighing between 180 to 220g were obtained from the animal house of the Department of Biochemistry, University of Calabar. They were housed in plastic cages in the animal house, and fed with rat pellets and tap water *ad libitum.* The animals were acclimatized for two weeks and their weights noted before the commencement of experimental treatment. They were then divided into seven groups of six rats each.

Group 1 served as the normal control and received 1ml of bottled water, group two received alcohol (2.5ml/kg body weight of Smirnoff vodka (40% v/v)), group three was given energy drink (5ml/kg body weight of power horse) while group four received paracetamol (28.55mg/kg body weight), group five received same dose of alcohol and energy drink, group six received same dose of alcohol and paracetamol, and group seven received same dose of alcohol, energy drink and paracetamol. The administration was carried out twice daily for 14 days.

At the end of the treatment period, the rats were weighed and fasted overnight. They were then anaesthetized with chloroform, dissected and their blood collected with sterile syringes by cardiac puncture into plane screw-cap bottles. They were left to clot for two hours and centrifuged in a MSE clinical centrifuge at 1000 rpm for 5 minutes. Serum was removed using a Pasteur pipette into another set of plane tubes for analysis.

ALT concentration in the samples was estimated by kit method of (Methew *et al.,* 1982). This is based on the catalytic transfer of the amino group from L-alanine to α-ketoglutarate forming pyruvate and glutamate. The enzyme concentration is determined from the rate of decrease of NADH measured at 340nm by means of the LDH coupled reaction.

AST activity in serum was estimated by the method of Mathew *et al.,* 1982 for the measurement of catalytic concentration of enzymes. It is based on the catalytic transfer of the amino group from Aspartate to α-ketoglutarate forming oxaloacetate and glutamate. The catalytic concentration is determined from the

rate of NADH decrease measured at 340nm by means of the malate dehydrogenase (MDH) coupled reaction.

The ALP activity in serum was estimated by kit method of Tietz, 1982 based on the measurement of the rate of hydrolysis of phosphate esters. Colourless P-Nitrophenol, which absorbs strongly at 405nm. The rate of increased absorbance at 405nm is proportional to the enzyme activity.

The data obtained were analysed statistically using analysis of variance (ANOVA) and the student's t-test at 95% (0.05) probability level.

RESULTS

The effect of administering energy drink and paracetamol during chronic alcohol consumption on the levels of Aspartate aminotransferease, Alanine aminotransferease, and alkaline phosphate in Wister albino rats was carried out. The result presented in (Table 1) shows a significant increase in AST activities in the groups treated with alcohol (50.25 ± 1.93), alcohol + energy drink (46.60 ± 4.55) compared to the control group (37.17± 0.93) at ($p< 0.05$) level.

However, non significant increases ($p > 0.05$) in the level of AST were observed in groups that received energy drink (37.40 ± 0.93), paracetamol (41.50 ± 1.78), alcohol+ paracetamol (39.25 ± 1.49) as well as the group treated with alcohol + energy drink + paracetamol (39.00 ± 0.63) compared to the normal control group (37.17 ± 2.09).

It was noticed that there was a non significant decrease ($p> 0.05$) in AST in the group treated with alcohol + energy drink (46.60 ± 4.55) compared to the alcohol group (50.25 ± 1.93); while groups administered with alcohol + paracetamol (39.25 ± 1.49) and alcohol+ energy drink + paracetamol showed a significant decreases ($p< 0.05$) in AST level compared to the group treated with alcohol alone.(50.25 ± 1.93).

Also, the group that received alcohol + energy drink (46.60 ± 4.55) showed a significant increase ($p < 0.05$) in AST compared to the energy drink group. However, the group treated with alcohol + energy drink + paracetamol (39.00 ± 0.63) showed non significant increase ($p> 0.05$) in AST compared to the group administered with only energy drink (37.40 ± 0.93). Non significant decreases ($p > 0.05$) were also observed in groups administered with alcohol + paracetamol (39.25 ± 1.49), as well as alcohol + energy drink + paracetamol (39.00 ± 0.63) compared to the paracetamol group (41.50 ± 1.78).

The level of serum alanine aminotransaminase (ALT) µmol/L were significantly increased ($p< 0.05$) in groups treated with alcohol (50.75 ± 0.85),alcohol + energy drink (41.20 ±3.06), alcohol+ energy drink + paracetamol (39.60 ± 1.96) respectively compared to the normal control group (32.67 ± 2.17).while there were non significant increase ($p> 0.05$) in the level of ALT in groups treated with energy drink (33.20 ± 1.69), paracetamol (36.33 ± 1.48) as well as the group that received alcohol + paracetamol (35.00 ± 2.94) compared to the normal control group (32.67 ± 2.17).

Also, Significant decreases ($p < 0.05$) were observed in the level of ALT in groups treated with alcohol + paracetamol (35.00 ± 2.94) , and alcohol+ energy drink + paracetamol (39.60 ± 1.63) compared to the group treated with alcohol (50.75 ± 0.85). However, the group that received alcohol+ energy drink + paracetamol (39.60 ± 1.96) showed a significant decrease ($p< 0.05$) in the level of ALT compared to the alcohol group.

The groups that received alcohol + energy drink (41.20 ± 3.06), and alcohol + energy drink + paracetamol (39.60 ± 1.96) had significantly increased ($p< 0.05$) ALT levels compared to the group that received only energy drink (37.40 ± 0.93).

There was no significant difference ($p> 0.05$) in the level of alkaline phosphatase in both the control and treatment groups. However, there were non significant increases ($p> 0.05$) in the level of ALP in the groups that received alcohol (69.00± 6.12) , energy drink (67.00 ± 4.72), paracetamol (70.83 ± 0.70), as well as alcohol + energy drink + paracetamol (68.40 ± 1.99) compared to the control group (65.50 ±

3.13). While a non significant reduction ($p > 0.05$) was observed in the group treated with alcohol + paracetamol (61.75 ± 2.06).

Table 1: Effect of administration of alcohol, energy drink, and paracetamol on serum enzymes levels

	AST(µmol/ L)	ALT (µmol/ L)	ALP (µmol/ L)
Gp. 1 (NC)	37.17±2.09	32.67±2.17	65.50±3.13
Gp. 2 (Alcohol)	50.25±1.93*	50.75±0.85*	69.00±6.12
Gp. 3 (Energy drink)	37.40±0.93[a]	33.20±1.69[a]	67.00±4.72
Gp. 4 (Paracetamol)	41.50±1.78[a]	36.33±1.48[a]	70.83±0.70
Gp.5 (Alc+Energy drink)	46.60±4.55*[, b]	41.20±3.06*[, b]	70.40±7.92
Gp. 6 (Alcohol+Paracetamol)	39.25±1.49[a, d]	35.00±2.94[a]	61.75±2.06
Gp. 7 (Alcohol + Energy drink +Paracetamol)	39.00±0.63[a, d]	39.60±1.96*[, a, b]	68.40±1.99

Values are expressed as mean ± SEM, n = 6.
**significantly different from NC at p<0.05*
a = significantly different from alcohol at p<0.05
b = significantly different from energy drink at p<0.05
d = significantly different from alcohol + energy drink at p<0.05

DISCUSSION

The commonest enzymes employed as indicators of hepatocellular damage are the transaminase enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as well as alkaline phosphate (ALT). Injury to the liver results in increase in their levels in plasma. Increases in these serum enzymes levels are roughly proportional to the extent of tissue damage (Klatsky *et al.,* 2006) .Elevations in the levels of aminotranferase enzymes are frequently the first findings during hepatocellular damage due to drugs and other toxic agents. These enzymes are released from hepatic cells when there is damage or increased cell permeability. Serum ezymes levels are particularly high in acute hepatocellular damage, chronic hepatocellular disease and in cholestatic conditions. A normal ALT level in the face of a raised AST levels confirms that liver pathology is not contributory to the raised enzymes level (Klatsky *et al., 2006*). In the presence inflammatory conditions, there is a leakage of cytoplasmic enzymes into the circulation; hence ALT level will rise above that of AST. Increased level of alkaline phosphatase (ALP) is associated with acute liver and kidney damage. All the treatment groups showed elevated levels of aspartate aminotransferase (AST) (umol/L) when compared to the normal control group. This shows that the consumption of alcohol, paracetamol, energy drink, paracetamol + energy drink, alcohol + paracetamol as well as the combination of alcohol + energy drink + paracetamol can cause the activity of the serum liver enzymes to increase.. It was also noticed that there was a non significant reduction in the group treated with alcohol + energy drink compared to the alcohol group. The groups administered with alcohol + paracetamol and alcohol+ energy drink + paracetamol showed significant decreases in AST level compared to the group treated with alcohol alone. Also, the group that received alcohol + energy drink showed a significant increase compared to the energy drink group. However, the group treated with

alcohol + energy drink + paracetamol showed a non significant increase (P > 0.05) compared to the group administered with only energy drink. The level of AST was non significantly lower (P> 0.05) in groups administered with alcohol + paracetamol, as well as alcohol+ energy drink + paracetamol compared to the group that was treated with paracetamol alone.

It was also observed that there was an increase in all the treatment groups of both alanine aminotransferase (ALT) and alkaline phosphate (ALP) (umol/ L) compared to the normal control. Increase in activity in liver disease are the result of increase synthesis of the enzymes by cells lining the bile canaliculi usually in response to cholestasis which may be intra or extra hepatic cholestasis even of short duration result in an increased enzyme activity to at least twice the upper end of the reference interval. Large increases in serum aminotransferase activity that is associated with small increase in alkaline phosphatase is indicative of hepatocellular damage, while small increases in the activity of the aminotransferase in plasma that is associated with large increase in alkaline phosphatase and gamma-glutamyl-transpeptidase activities is indicating a biliary obstruction (Klatsky *et al.*, 2006). Large increases in serum aminotransferase activity that is associated with small increase in alkaline phosphate (ALP) is indicative of hepatocellular damage, while small increases in the activity of the aminotransferase in plasma that is associated with large increase in alkaline phosphate and gamma-glutamyl-transpeptidase activities is indicative of a biliary obstruction

Conclusion

In conclusion, it is observed that paracetamol administration during sub-chronic alcohol consumption as well as consumption of energy drink, individually or in combination with paracetamol results in improved liver enzymes outcome compared to the consumption of alcohol alone.

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