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

ACETAMINOPHEN AND CAFFEINATED ENERGY DRINKS EFFECT ON BODY WEIGHT AND SERUM BILIRUBIN LEVEL EXPOSED TO CHRONIC ALCOHOL CONSUMPTION IN WISTAR ALBINO RATS

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ABSTRACT
The effect of administering acetaminophen and energy drink on body weight and Serum bilirubin during 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 show that, there was no significant difference ($p<0.05$) in the initial and final body weight of the rats in the normal control group. However, alcohol caused a significant decrease ($p<0.05$) in both percentage growth rate and percentage body weight compared to control and other treatment groups. Also, significant increases ($p< 0.05$) in bilirubin levels in the groups receiving alcohol (9.65±0.33), and alcohol +energy drink+ paracetamol (13.16±0.41) compared to the normal control group (5.80±0.20) was observed. Comparison of total billirubin (µmol/L), conjugated and unconjugated billirubin levels in Wister albino rats treated with alcohol, alcohol +energy drink+ paracetamol and alcohol +energy drink with those in the normal control group showed an increase in total billirubin, conjugated and unconjugated billirubin level after treatment for 21 days compared with the normal control group. Therefore, heavy drinking of alcohol may be constitute health hazard and should be avoided.

Keywords: *Acetaminophen, Caffeinated Energy Drinks Body Weight and Serum Bilirubin level*

INTRODUCTION
Alcoholic beverage is created when grains, fruits, or vegetables are fermented**.** It is a depressant and slows the function of the central nervous system. It actually blocks some of the messages trying to get to the brain. This alters a person's perceptions, emotions, movement, vision, and hearing (Jones–Webb, 1998). Alcoholic beverages are divided into three general classes: beers, wines, and spirits .They all contain different percentage of alcohol. When large amounts of alcohol are consumed in a short period of time, alcohol poisoning result, this is a process whereby the body becomes poisoned by large amounts of alcohol. Violent vomiting is usually the first symptom of alcohol poisoning. Extreme sleepiness, unconsciousness, difficulty in breathing, dangerously low blood sugar, seizures, and even death may result (Smith *et al*, 2006).

Due to the toxic effect of alcohol, people tend to mix it with energy drink in order to reduce the level of intoxication. The Caffeine in energy drink is a central nervous system stimulant that temporarily increases attention, alertness and motor activity, while alcohol is a depressant, which tends to slow down brain and motor activity. Individually, the two substances serve completely opposite functions. However, in combination, they can magnify negative effects in the body such as increased heart rate, blood pressure, headache and urine elimination (Shapiro and Robert, 2008). Also, Short term side effects such as headache, nausea, and anxiety have been shown as symptoms of mild caffeine consumption (Ferreira *et*

al, 2006). These energy drinks claim to stimulate the mind and body, provide a boost of energy but can have adverse effects when mixed with alcohol. However, power horse for instance, is an energy drink which contains up to 80 mg or more of caffeine per can (O'Brien *et al*, 2008).

High levels of caffeine can boost heart rate and blood pressure, causing palpitations. Mixing these drinks with alcohol further increases the risk of heart rhythm problems. It has also been reported that although energy drinks have stimulants in it, the alcohol still has similar effects. Energy drinks have a lot of stimulants in them like ginseng, since alcohol is a depressant, in mixing the two; a mixed message is being sent to the nervous system which can cause cardiac related problems (Alford *et al.*, 2001). Alcohol makes people dehydrated, which is one of the reasons why people have hangovers, and the caffeine in the energy drinks is a diuretic which also causes loss of water, thereby worsening the effects of dehydration (Alford *et al.*, 2001). Paracetamol is one of the drugs used as a hangover cure, by millions of people worldwide but mixing caffeine in the energy drink with paracetamol could be deadly (Emby and Fraser, 1977). Combining large quantities of the pain-killer and caffeine in the energy drink appeared to increase the risk of liver damage. Also it has been shown that Caffeine in the energy drink tripled the amount of a toxic by-product created when paracetamol is broken down (Jaya *et al.*, 1994).

It has also been shown that combining coffee with paracetamol could also prove deadly (Nelson, 2005). However, some people would be more susceptible, such as those taking anti-epilepsy medicines, or St John's wort, a herbal antidepressant as both of these boost levels of the enzyme involved. Alcoholic beverages are widely consumed in different forms and in a large quantity. Due to its toxic and depressant effect, people who drink now mix the alcoholic beverage with energy drinks. This Energy drinks on their own contain some amount of caffeine, from high to low mg of caffeine depending on the type of energy drink (Childs and de Wit, 2008). The reason for the mixture, being to reduce the depressant 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 (Scholey and Kennedy, 2004).This large intake of alcohol leads to a hangover, and without considering the effect of this mixture, paracetamol is taken to reduce the headache and other problems associated with alcoholic hangover.

However, the reason why caffeine, alcohol and paracetamol may be so toxic together isn't fully understood, hence the reason for this research. But the combination appears to impair a drinker's judgment more than taking alcohol alone. Amongst some users, there is a higher incidence of risk-taking behaviors because the perceptions of their limitations are distorted, (Scholey and Kennedy, 2004). As a stimulant, caffeine stimulates whole body, increasing blood pressure, heart rate and in some cases, it can cause heart palpitation. Caffeine also leads to headaches, jitteriness, agitation, stomach problems and abnormal breathing. This is the equivalence of an adrenaline rush.

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.

Laboratory 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 fasted overnight, and anaesthetized with chloroform. They were then dissected and their blood collected with sterile syringes by cardiac puncture. The blood was collected into plane screw-cap bottles for biochemical analysis. The samples were centrifuged at 700rpm for 15 minutes using an MSE table top centrifuge. Serum was collected using a semi-automatic pipette into labeled specimen tubes. The serum was stored in a refrigerator until when required for analysis. The storage period however did not exceed 48 hours. Beakers, centrifuge-tubes, orogastric tubes, pipettes, heparized screw-cap-tube, fume chamber MSE centrifuge (England), cuvettes and haematocrit reader, light microscope, spectrophotometer (DRE 3000 HACH spectrophotometer).

Growth rate

This was calculated as the ratio of the weight gained during the treatment period to the number of days constituting the treatment period, and was presented as percentage growth rate.

Weight increase

Initial and final weight of each rat was measured and the mean weight for each group was determined. Weight increase was calculated as the ratio of change in weight during the treatment period to the initial weight and was presented as percentage weight increase.

Statistical analysis

The data obtained were analysed statistically using analysis of variance (ANOVA) and the student's t-test to determine whether or not the null hypothesis should be rejected so as to accept the alternative hypothesis corresponding at 95% (0.05) probability level.

RESULT

Effect of administration of acetaminophen, energy drink and alcohol on the body weight of wistar albino rats

The weight of rats after 14 days of administration of alcohol, energy drink and paracetamol were obtained and compared with the initial weight measured before treatment. The differences in weights were used to determine the percentage weight increase and the growth rate during this period. The results show significant differences ($P < 0.05$) in all the treatment groups compared to the normal control group. However, there were significant decreases ($P < 0.05$) in both percentage growth rate and percentage body weight in group treated with energy drink (-(142.9 ± 3.65 and -11.10 ± 3.86) compared to the group treated with alcohol (-226.2 ± 0.21 and-16.97 ± 0, 22). Also, the group treated with paracetamol (Acetaminophen) (47.6 ± 7.92 and 4.08 ± 7.91) was the only group that showed positive values and the values obtained reduced significantly ($P< .05$) compared to groups treated with alcohol and energy (-226.2 ± 0.21 and -16.97 ± 0, 22). The group administered with energy drink + alcohol (-142.9 ± 4.21 and -11.91 ± 8.43) showed significant decreases ($P> 0.05$) in both percentage body weight percentage growth rate compared to the group treated with paracetamol (47.6 ± 7.92 and 4.08 ± 7.91). Significant decreases ($P< 0.05$) were also observed in groups treated with Energy drink + paracetamol (-142.9 ± 4.21 and -11.91 ± 8.43) compared to the group treated with only alcohol (-226.2 ± 0.21 and -16.97 ± 0.22). Nevertheless, there was a non significant difference ($P> 0.05$) in the percentage growth rate and percentage body weight of rats treated with only energy drink (-142.9 ± 3.65 and -11.10 ± 3.86) compared to the group receiving energy drink + alcohol (-142.9 ± 4.21 and -11.91 ± 843). Administration of alcohol + paracetamol (-119.0 ± 3.52 and 10. 04 ± 3,51) on Wister albino rat cause a significant decrease ($P < 0.05$) in both percentage body weight and percentage growth rate compared to the group

treated with alcohol (-226.2 ± 0.21and -16.97 ± 0.22) and paracetamol (47.6 ± 7.92 and 4.08 ± 7.91) respectively.

However, the group administered with alcohol + energy drink + paracetamol (-(171.4 ± 4.94 and 10.91 ± 4.31) showed a significant decrease($P < 0.05$) in both percentage growth rate and percentage body weight of rats compared to the groups treated with alcohol (-226.2 ± 0.21 and -16.97 ± 0,22),energy drink (-142.9 ± 3.65 and 11.10 ± 3.86) as well as paracetamol (47.6 ± 7.92 and 4.08 ± 7.91).

Effect of administration of alcohol, energy drink, and paracetamol on serum bilirubin levels (µmol/L)

From the result, there were significant increases ($p< 0.05$) in bilirubin levels in the groups receiving alcohol (9.65±0.33), and alcohol +energy drink+ paracetamol (13.16±0.41) compared to the normal control group (5.80±0.20). However, the group treated with energy drink (4.72 ± 0.18) showed a significant decrease ($p < 0.05$) in bilirubin level compared to the normal control group. Nevertheless, there were non significant decreases ($p > 0.05$) in groups treated with paracetamol (5.58± 0.18) and alcohol+ paracetamol (5.65±0.27) compared to the normal control group. Also, the group administered with alcohol and energy drink (6.22±0.12) showed a non significant increase ($p> 0.05$) compared to the normal control group.

The level of total bilirubin significantly increased ($p< 0.05$) in the group treated with alcohol+energy drink + paracetamol (13.16 ± 0.41) compared to the group that received alcohol. There were also significant decreases ($p < 0.05$) in groups administered with alcohol + energy drink (6.22±0.12) and alcohol + paracetamol (5.65± 0.27) compared to the alcohol group (9.65 ± 0.33).However, there were significant increases ($p < 0.05$) in groups that were treated with alcohol + energy drink (6.22±0.27) and alcohol+ energy drink +paracetamol (13.16 ± 0.41) compared to the group that received energy drink (4.72 ± 0.18). The group treated with alcohol +paracetamol (5.65 ± 0.27) also showed a non significant increase ($p > 0.05$) compared to the paracetamol group (5.58 ± 0.18). And there was a significant increase ($p< 0.05$) in the group that received alcohol + energy drink + paracetamol (13.16 ± 0.41) compared to the paracetamol group (5.58± 0.18). However, the values obtained for total bilirubin in both control and test groups were above the reference range of 0.1-1.0 µmol/L (Toa and Viska 2007)

Effect of alcohol, energy drink and paracetamol on conjugated bilirubin

The conjugated bilirubin level (µmol/L) showed a significant increases ($p< 0.05$) in groups treated with alcohol (5.73 ± 0.19), and alcohol+ energy drink + paracetamol (6.90 ± 0.17) compared to the normal control group (4.18 ± 0.22). There was also a significant decrease ($p <0.05$) in the level of conjugated bilirubin in the group that received energy drink (3.08 ± 0.10).compare to the normal control group. However, groups administered with paracetamol (3.73 ± 0.22) and alcohol + paracetamol (3.93 ± 0.31) showed no significant decreases ($p> 0.05$) in the level of conjugated bilirubin compared to the normal control group. There was also a non significant increase ($p>0.05$) in conjugated bilirubin level in the group treated with alcohol + energy drink (4.64 ± 0.04) compared to the normal control group.

The groups treated with alcohol + energy drink and alcohol + paracetamol (4.64 ± 0.04, 3.93 ± 0.31) respectively showed significant decreases ($p < 0.05$) in the level of conjugated bilirubin compared to the alcohol group (5.73 ± 0.19). There was a significant increase ($p < 0.05$) in the group that received alcohol+ energy drink + paracetamol (6.90 ± 0.17) compared to the alcohol group (5.73 ± 0.19).

Nevertheless, there were significant increases ($p < 0.05$) in groups administered with alcohol + energy drink (4.64 ± 0.04) and alcohol + energy drink + paracetamol (6.90 ± 0.17) compared to the group treated with energy drink (3.08 ± 0.10). Also, the group treated with alcohol + paracetamol (3.93 ± 0.31) showed a non significant increase ($P > 0.05$) compared to the paracetamol group (3. 73 ± 0.22). And there was also a significant increase ($p< 0.05$) in group that received alcohol + energy drink + paracetamol (6.90 ± 0.17) compared to the group treated with only paracetamol (3. 73 ± 0.22). However, the values obtained for conjugated bilirubin in both control and test groups were within the reference range of 5-7µmol/L(upper university hospital)

Table 1: Effect of administration of acetaminophen, energy drink and alcohol on the body weight

Group	Treatment	Growth rate %	Weight increase %
1	Normal control	62.53 ± 2.14	4.38 ± 1.88
2	Alcohol	-226.20 ± 0.21*	-16.97 ± 0.22*
3	Energy drink	-142.90 ± 3.65*,a	-11.10 ± 1.86*,a
4	Paracetamol	47.60 ± 2.92*,a,b	4.08 ± 1.91*, a,b
5	Alcohol + energy drink	-142.90 ± 4.21*,a,c	-11.91 ± 1.43*,a,c
6	Alcohol + paracetamol	-119.0 ± 3.52*,a,b,c,d	-10. 04 ± 1.51*, a,c
7	Alcohol+energydrink+ Paracetamol	-171.40 ± 4.94*,a,b,c,d,e	-10.91 ± 1.31*,a,c

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
c = significantly different from paracetamol at p<0.05
d = significantly different from alcohol + energy drink at p<0.05
e = significantly different from alcohol + energy drink + paracetamol at p<0.05

Table 2: Effect of administration of acetaminophen, energy drink and alcohol on Total bilirubin, Conjugated bilirubin and Unconjugated bilirubin

Treatment groups	Tot. bilirubin (µmol/L)	Conjugated Bilirubin (µmol/L)	Unconjugated Bilirubin (µmol/L)
Gp. 1 NC	5.80±0.20	4.18±0.22	1.62±0.11
Gp. 2 Alcohol	9.65±0.33*	5.73 ±0.19*	3.93±0.17*
Gp. 3 Energy drink	4.72±0.18*, a	3.08±0.10*, a	1.64±0.13a
Gp. 4 Paracetamol	5.58 ±0.18a, b	3.73±0.22a, b	1.85 ±0.12a
Gp.5Alcohol+E. drink	6.22 ±0.12a, b	4.64±0.04a, b, c	1.58±0.12a
Gp6:Alcohol+Paracetamol	5.65±0.27a, b	3.93±0.31a, b, d	1.73±0.06a
Gp.7Alc+E.drink+Paracetamol	13.16±0.41*,a,b,c,d,e	6.90±0.17*,a,b,c,d,e	6.26±0.34*,a,b,c,d,e

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
c = significantly different from paracetamol at p<0.05
d = significantly different from alcohol + energy drink at p<0.05
e = significantly different from alcohol + energy drink + paracetamol at p<0.05

Effect of alcohol, energy drink and paracetamol on unconjugated billirubin

The group treated with alcohol (3.93 ± 0.17) and alcohol+ energy drink + paracetamol (6.26 ± 0.34) compared to the normal control group (1.62 ± 0.11).However, there were no significant increases (p >

0.05) in the groups administered with energy drink (1.64 ± 0.13), paracetamol (1.85 ± 0.12) and alcohol + paracetamol (1.73 ± 0.06) respectively compared to the normal control group (1.62 ± 0.11). Also, the group treated with alcohol + energy drink (1.58 ± 0.12) showed a non significant decrease (p> 0.05) in the level of unconjugated bilirubin compared to the normal control group.

There were significant decreases (p< 0.05) in unconjugated bilirubin level in the groups treated with alcohol + energy drink (1.58 ± 0.12) and alcohol + paracetamol (1.73 ± 0.06) compared to the alcohol group (3.93 ± 0.17) .However, the group treated with alcohol + energy drink + paracetamol (6.26 ± 0.34) showed a significant increase (p< 0.05) in the level of unconjugated bilirubin compared to the group treated with alcohol (3.93± 0.17).

Also, the group that received alcohol + energy drink (1.58 ± 0.12) showed a non significant decrease (p > 0.05) in the level of unconjugated bilirubin compared to the energy drink group. Nevertheless, there was a significant increase (p < 0.05) in the group treated with alcohol + energy drink + paracetamol (6.26 ± 0.34) compared to the group treated with energy drink (1.64 ± 0.13)

Also, the group that received alcohol + paracetamol (1.73 ± 0.06) showed a non significant decrease (p> 0.05) in the unconjugated bilirubin level compared to the group that received only paracetamol (1.85 ±0.12). However, there was a significant increase (p < 0.05) in the level of unconjugated bilirubin in the group treated with alcohol+ energy drink + paracetamol (6.26 ± 0.34) compared to the group treated with paracetamol alone

DISCUSSION

From the result, the initial and final weight of the albino Wister rats were the same in the control group, thereby causing both the percentage growth rate and the percentage weight values to be zero (0). Therefore there were significant differences (P< 0.05) in all treatment groups compared to the normal control group. It was observed that the group treated with alcohol had the lowest value of both percentage growth rate and percentage body weight increase compared to all other treatment groups. This high reduction could have been due to malnutrition and absence of some vital nutrients. This is in line with an earlier report which says that, although alcoholic beverages contain calories, and under certain conditions these calories do not have as much value for the body as those derived from other nutrients (Pirola *et al*, 1972**).** Lodgsdon, 1994 also reported that, Alcoholism is a major cause of malnutrition. This is because; alcohol interferes with central mechanisms that regulate food intake and causes food intake to decrease. Increasing amounts of alcohol ingested can therefore lead to the consumption of decreasing amounts of other foods, making the nutrient content of the diet inadequate, even if total energy intake is sufficient. Thus chronic alcohol abuse causes primary malnutrition by displacing other dietary nutrients.

However, apart from alcohol group, the group receiving Alcohol+ energy drink + paracetamol showed a significant reduction (P< 0.05) in both percentage growth rate and percentage body weight increase compared to all other treatment groups. This could have been due to the combine effect of energy drink, alcohol and paracetamol according to Jaya *et al, 1994.* The group administered with paracetamol was observed to have the highest value of both percentage growth rate and percentage body weight increase. Bilirubin is the yellow breakdown product of normal heme catabolism and it is excreted in bile and urine. For many years, the bile pigment bilirubin was considered to be only a toxic waste product formed during heme catabolism. Recent evidence, however, suggests that bilirubin acts as a potent physiologic antioxidant that may provide important protection against arteriosclerosis, coronary artery disease (CAD), and inflammation (Yamaguchi *et al*, 1996). Levels of billirubin may indicate certain diseases .Some of the diseases caused by elevated level of billirubin are: Unusually large bile duct obstruction, e.g. stone in common bile duct, tumour obstructing common bile duct, severe liver failure with cirrhosis etc. Cirrhosis may cause normal, moderately high or high levels of bilirubin, depending on exact features of the cirrhosis system (Jaya *et al,* 1994).Comparison of total billirubin (µmol/L), conjugated and unconjugated billirubin levels in Wister albino rats treated with alcohol, alcohol +energy drink+ paracetamol and

alcohol +energy drink with those in the normal control group showed an increase in total billirubin, conjugated and unconjugated billirubin level after treatment for 21 days compared with the normal control group. This agrees with the earlier researchers that, heavy drinking of alcohol is associated with liver disease, such as cirrhosis (Takase *et al*., 1993), and that drinking alcohol with caffeine mixed with acetaminophen all produces metabolites which are extremely toxic and harmful to the liver as well as the entire nervous

However, Bilirubin is more than just a blood by-product, it acts like an antioxidant and anti-inflammatory. Low bilirubin levels are associated with coronary artery disease, inflammation and arteriosclerosis. Thus, decreased bilirubin levels are associated with angina, which is caused by coronary artery disease and arteriosclerosis as well as inflammation. In this research it was observed that there was a decrease in the level of total bilirubin , conjugated as well as unconjugated billirubin in the groups treated with energy drink, paracetamol , alcohol + paracetamol and alcohol + energy drink. This reduction is assumed to be a symptom coronary artery disease , also stimulant effects of caffeine in energy drink may increase the amount of blood reaching the heart through the coronary arteries because of vasodilation, however, stimulant effect of energy drink speeds up the heart rate, the heart needs more oxygen and this can counteract the effects the vasodilation (Ferreira *et al.,* 2006) .

CONCLUSION

Chronic alcohol consumption was seen to cause a significant drop in the body weight. Also**,** total billirubin (µmol/L), conjugated and unconjugated billirubin levels in Wister albino rats treated with alcohol, alcohol +energy drink+ paracetamol and alcohol +energy drink led to an increase in total billirubin, conjugated and unconjugated billirubin. Which could cause the production of metabolites which are extremely toxic and harmful to the liver as well as the entire nervous system therefore this combination might be dangerous to health.

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