International Journal of Basic and Applied Chemical Sciences ISSN: 2277-2073
Online, International Journal Available at http://www.cibtech.org/jcs.htm
2020 Vol.10, pp.1-8/Ukpanukpong et al.
***Research Article** (Open Access)*

EFFECT OF ACETAMINOPHEN AND CAFFEINATED ENERGY DRINK ON THE SERUM BILLIRUBIN CONCENTRATION, CREATINE AND UREA LEVELS OF WISTAR ALBINO RATS DURING SUB-CHRONIC ALCOHOL CONSUMPTION

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ABSTRACT
The effect of administering acetaminophen and energy drink on Billirumin concentration, as well as serum creatine and urea levels during sub-chronic alcohol consumption was investigated on 42 Wister albino rats that 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), group three was given energy drink (5ml/kg body weight), group four received paracetamol (28.55mg/kg body weight), group five received same dose of alcohol, energy drink, group six received same dose of alcohol and paracetamol, group seven received same dose of alcohol, energy drink paracetamol. The administration was twice daily for 14 days. From the result, Billirubin concentration was significantly higher in the group administered with paracetamol+ energy drink + alcohol compared to all other groups. Also from the result, level of urea reduced significantly ($p<0.05$) in the groups treated with alcohol (6.28 ± 0.67), alcohol + energy drink (5.22 ± 0.70), alcohol + paracetamol (5.28 ± 0.23) as well as alcohol + energy drink + paracetamol (5.38 ± 0.10) compared to the normal control group (7.62 ± 0.31). The group treated with alcohol (72.95 ± 3.69) showed a significant decrease ($p<0.05$) compared to the normal control group (89.48 ± 0.66). While the groups treated with energy drink (81.30 ± 4.92), alcohol + paracetamol (82.45 ± 3.47), as well as alcohol + energy drink + paracetamol (81.16 ± 2.44) showed non significant reductions ($p>0.05$) in the level of creatinine.

Keywords: *Alcohol; Billirubin; Creatinine; ConJugated Billirubin; Energy Drink; Unconjugated Billirubin; Urea, Wistar Albino Rat*

INRODUCTION
Alcoholic beverage is created when grains, fruits, or vegetables are fermented**.** It is a depressant**,** which means it 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 (Shapiro and Robert, 2008). 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 slows 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 causes dehydration, 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, there by 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 (Nelson, 2005). 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 toxic by-product created when paracetamol is broken down (Roland *et al.*, 2007). Caffeine addicts are being warned against mixing the drink with paracetamol because caffeine can react with the painkiller to cause liver damage. The effect could be fatal for susceptible people if taken in large amount. Scientists have also shown that combining coffee with the drug 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. Those who drink a lot of alcohol are also at higher risk, while people should be aware that many paracetamol-based painkillers also contain caffeine (Nelson, 2005).

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 weighed and fasted overnight. They were then anaesthetized with chloroform, dissected and their blood collected with sterile syringes by cardiac puncture into heparinized screw-cap bottles for haematological analysis.

Estimation of urea concentration

PROCEDURE

The working solution was prepared by pipetting 10µ/l of the sample and standard into 1000µ/l of the working reagent. This was mixed and incubated for 10 minutes at 15-25^{o}C before adding reagent 2. The final mixture was then incubated for 10 minutes at 15-25^{o}C and the extinction measured at 580nm against the reagent blank. The concentration of urea (mg/dl) was calculated as the ratio of the change in the absorbance of the sample and standard multiplied by the standard concentration.

Estimation of creatinine concentration

PROCEDURE

Reagent and samples were brought to room temperature. 1000µ/l of the sample was mixed with 1000µ/l of reagent 1 and incubated for 5 minutes at 20-25^{o}C, then reagent 2 was added. The instrument was zeroed with reagent blank, the mixture incubated for 5 minutes and read as A2. The concentration of creatinine (mg/dl) was calculated as the ratio of the change in the absorbance of the sample and standard multiplied by the standard concentration.

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

Serum billirubin concentration

From the result, there were significant increases ($p<0.05$) in billirubin 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 billirubin 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 billirubin 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 billirubin in both control and test groups were above the reference range of 0.1-1.0 µmol/L (Toa and Viska, 2007).

Conjugated billirubin

The conjugated billirubin 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 billirubin 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 billirubin compared to the normal control group. There was also a non significant increase ($p>0.05$) in conjugated billirubin 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 billirubin 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 billirubin in both control and test groups were within the reference range of 5-7µmol/L (Toa and Viska, 2007).

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 billirubin compared to the normal control group.

There were significant decreases ($p< 0.05$) in unconjugated billirubin 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 billirubin 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 billirubin 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 billirubin 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 billirubin in the group treated with alcohol + energy drink + paracetamol (6.26 ± 0.34) compared to the group treated

Serum urea level

The effect of administering energy drink and paracetamol during chronic alcohol consumption in the urea and creatinine levels in Wistar albino rats was investigated. The results show that ,the level of urea reduced significantly ($p<0.05$) in the groups treated with alcohol (6.28 ± 0.67), alcohol + energy drink (5.22 ± 0.70), alcohol + paracetamol (5.28 ± 0.23) as well as alcohol + energy drink + paracetamol (5.38 ± 0.10) compared to the normal control group (7.62 ± 0.31).However, non significant reductions ($p>0.05$) in the level of serum urea were noticed in groups that were treated with energy drink (7.36 ± 0.33), and paracetamol (7.47 ± 0.21) compared to the normal control group.

Also the groups administered with alcohol + energy drink (5.22 ± 0.70), alcohol + paracetamol (5.28 ± 0.23), and alcohol + energy drink + paracetamol (5.38 ± 0.10) showed non significant decreases ($p> 0.05$) in the level of serum urea compared to the alcohol group. However, serum urea mmol/L level showed significant decreases ($p< 0.05$) in both groups treated with alcohol+ energy drink (5.22 ± 0.70) , and alcohol + energy drink + paracetamol(5.38 ± 0.10) compared to the group treated with energy drink (7.36.± 0.33).There were also significant reductions ($p<0.05$) in the level of serum urea in groups that received alcohol + paracetamol (5.28 ± 0.23), and alcohol+ energy drink + paracetamol (5.38 ± 0.10) compared to the group that received paracetamol (7.47 ± 0.21).

Creatinine level

For creatinine (µmol/L), the group treated with alcohol (72.95 ± 3.69) showed a significant decrease ($p<0.05$) compared to the normal control group (89.48 ± 0.66). While the groups treated with energy drink (81.30 ± 4.92), alcohol + paracetamol (82.45 ± 3.47), as well as alcohol + energy drink + paracetamol (81.16 ± 2.44) showed non significant reductions ($p>0.05$) in the level of creatinine, compared to the normal control group (89.48 ± 0.66). There were no significant increases ($p>0.05$) in the

level of creatinine in groups administered with paracetamol (89.53 ± 4.55) and alcohol + energy drink (94.00 ± 2.84) compared to the normal control group (89.48 ± 0.66). Also, there was a significant increase ($p< 0.05$) in the level of creatinine in the group administered with alcohol + energy drink (94.00 ± 2.84) compared to the alcohol group (72.95 ± 3.69). While there were non significant increases ($p>0.05$) in creatinine levels in groups treated with alcohol + paracetamol (82.45 ± 3.47), and alcohol + energy drink + paracetamol (81.16 ± 2.44) compared to the alcohol group (72.95 ± 3.69).
However, the group that was administered with alcohol and energy drink (94.00 ± 2.84) showed a significant increase ($p<0.05$) compared to the group treated with energy drink (81.30 ± 4.92). While the group that received alcohol + energy drink + paracetamol (81.16 ± 2.44) was observed to have a non significant decrease ($p>0.05$) in the level of creatinine compared to the energy drink group (81.30 ± 4.92). Also, there were non significant decreases ($p>0.05$) in creatinine level in both the group treated with alcohol+ paracetamol (82.45 ± 3.47), and the group treated with alcohol + energy drink + paracetamol (81.16 ± 2.44) compared to the paracetamol group (89.53 ± 4.55). However, the values obtained for creatinine in both control and test groups were within the reference range of 50-98 (Finney and Newman 2000).

Table 1: Effect of administration of alcohol, energy drink, and paracetamol on serum bilirubin concentrations

Treatment Group	Total Bilirubin (µmol/L)	Conjugated Bilirubin (µmol/L)	Unconjugated Bilirubin (µmol/L)
NC	5.80±0.20	4.18±0.22	1.62±0.11
Alcohol	9.65±0.33*	5.73±0.19*	3.93±0.17*
Energy drink	4.72±0.18*, a	3.08±0.10*, a	1.64±0.13a
Paracetamol	5.58±0.18a, b	3.73±0.22a, b	1.85±0.12a
Alcohol + Energy drink	6.22±0.12a, b	4.64±0.04a, b, c	1.58±0.12a
Alcohol+Paracetamol	5.65±0.27a, b	3.93±0.31a, b, d	1.73±0.06a
Alcohol + 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

Table 2: Effect of administration of alcohol, energy drink, and paracetamol on serum urea and creatinine concentrations.

Treatment Group	Urea	Creatinine
Normal Control	7.62±0.31	89.48±0.66
Alcohol	6.28±0.67*	72.95±3.69*
Energy drink	7.36±0.33	81.30±4.92
(Paracetamol)	7.47±0.21	89.53±4.55[a]
Alcohol + Energy drink	5.22±0.70*[, b, c]	94.00±2.84[a, b, c]
Alcohol + Paracetamol	5.28±0.23*[, b, c]	82.45±3.47[d]
Alcohol + E.drink +Paracet.	5.38±0.10*[, b, c]	81.16±2.44[d]

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

DISCUSSION

Decrease in blood urea levels may be seen in some cases of severe liver damage and physiological conditions Chatterjea and Shinde, (2002). From the result there were significant reduction in level of urea in the groups treated with alcohol, alcohol + energy drink, alcohol + paracetamol as well as alcohol + energy drink + paracetamol compared to the normal control group. However, serum urea (mmol/L) level showed a significant reduction in both groups treated with alcohol+ energy drink, and alcohol + energy drink + paracetamol compared to the group treated with energy drink alone. This therefore supports an earlier report by Chatterjea and Shinde, (2002). Alcohol + paracetamol, and alcohol+ energy drink + paracetamol also produce a reduction in serum urea level (mmol/L) when compared to the group that received paracetamol alone. This reduction was assumed to be caused by the toxic effect of alcohol and paracetamol which affected the liver leading to liver damage. This confirms the earlier report that, Low serum urea concentrations were encountered in a high proportion of patients after paracetamol overdose. This is likely to be explained by a high prevalence of risk factors for hepatotoxicity. Chronic ethanol excess is also associated with low serum urea regardless of whether there is any established liver disease (Lum and Leal-Khouri, 1989). Elevated serum creatinine has been associated with increased mortality in hypertensive persons, the elderly, and patients with myocardial infarction or stroke in whom cardiovascular disease is the major cause of death (Volpe *et al., 1997).* Renal impairment (acute and chronic) and high muscle mass are the major causes of high level of creatinine. Decreased creatinine levels may also be seen in the elderly, persons with small stature, decreased muscle mass or inadequate dietary protein. While normal creatinine levels indicates normal kidney functioning. The values obtained for creatinine in both control and test groups were observed to be within the reference range of 50-98 (Finney and Newman 2000). But when compared to other treatment groups, the group treated with alcohol and energy drink showed a high level of creatinine which was in line with earlier report by O'Brien *et al.*, (2008).

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 is: 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 (Kuntz, and Erwin, 2008). Comparison of total billirubin (µmol/L), conjugated and unconjugated billirubin levels in Wistaralbino 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 system (Jaya *et al.*, 1994).

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

In conclusion, the consumption of Energy drink, paractamol , Energy drink with combination with alcohol , and the combination of the three, energy drink, alcohol and paracetamol can lead to an onset of coronary artery disease. Also the toxic effect of paractamol and alcohol can lead to liver damage, while the stimulating effect caused by the caffeine which is a stimulant in energy can increase heart rate.

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