International Journal of Medicine Research
ISSN: 2455-7404; Impact Factor: RJIF 5.42
www.medicinesjournal.com
Volume 2; Issue 1; January 2017; Page No. 11-16

Alcohol and energy drink: a safe mix?

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Abstract
The intake of alcoholic beverages has an important social and historical role and the consequences of its use and abuse are still a reality in the contemporary society. It is related to 30% to 50% of serious and fatal traffic accidents in several countries. Recently it has been observed the popularization of the use of alcoholic beverages in association with energy drinks. Due to its stimulating properties, energy drinks have become very popular especially among young people in Brazil and around the world. Due to the high consume of energy drinks associated or not with alcohol, the aim of this study was to evaluate the effects of using alcohol, energy drink and the mixture of these two drinks in the metabolic profile and behavioral parameters of Wistar rats. Male rats were divided in G1 (control group that was fed and watered), G2 (treated with *cachaça* mixed with water), G3 (treated with energy drink and water 1:1) and G4 (treated with cachaça and energy drink 1:1). After a period of 45 days, the animals performed the Elevated Plus Maze and after that they were euthanized and blood samples were collected to evaluations of total cholesterol, HDL-c, triglycerides, glycaemia, AST, ALT and CPK. Atherogenic Coefficient (AC), Atherogenic Index (AI), Cardiac Risk Ratio 1 (CRR1), and non-HDL-c levels were also analyzed. Our results regarding food consumption, body parameters, glycaemia, HDL-c, AST, ALT, CPK, AC, CCR1 and non-HDL-c revealed no significant differences among the groups. Significant increase were found for AI, triglycerides and total cholesterol levels in the G2, G3 and G4. Results for behavioral test showed that the use of the drinks significantly modify the frequency in the open and closed arm and in the center. We may conclude that the consumption of alcohol, energy drinks and the association of these beverages may increase the risk for cardiovascular diseases once they increase Atherogenic Index and levels of cholesterol and triglycerides.

Keywords: Alcohol, energy drinks, lipids, atherogenic indices

1. Introduction

The consumption of alcoholic beverages has an important social and historical role. Configuring itself as a socially accepted drug, it is seen as a facilitator of social interactions and for this reason, alcohol has always been present in the most distinct social and historical spheres [1, 5].

Not only a problem of ancient societies, the consequences of alcohol use and abuse are still a reality in contemporary society. Being the cause of 30% to 50% of serious and fatal traffic accidents in several countries, alcohol is among the recreational drugs most used by humanity [6, 8].

Over time, the use of alcoholic beverages was associated with several substances, with the aim of depressing or masking the symptoms of intoxication. Recently it has been observed the popularization of the use of alcoholic beverages, especially distilled, in association with energy drinks [9, 11].

Energy drinks that are composed mainly of taurine and caffeine have the main function of increasing energy levels, through the stimulation of metabolism and usually contains vitamin B, methylxanthines, caffeine, ginkgobiloba, glucoronolactone, creatine, maltodextrin, taurine, inositol, guarana and ginseng [12, 16].

From the components of the energy drinks, caffeine acts as a stimulant in the central nervous system, and enhances brain activity resulting in increased attention, due to the release of adrenaline and calcium. Taurine helps in physical endurance and also decreases the consequences caused by post-alcohol depression. Glucoronolactone, a glucose-based substance, helps in the elimination of exogenous and endogenous toxins and increases physical performance and decrease fatigue [9, 15, 17, 19].

Due to its stimulating properties, energy drinks have become very popular and its association with alcoholic beverages has become very common, especially among young people in Brazil and around the world. Alcoholic beverages have strong flavor and the addition of energetic drinks, due to the sweet taste, make alcohol easier to consume [20, 21].

Epidemiologic studies point that the prevalence of energy drinks consume among students and other young adults may vary from 38 to 48% [22]. Other studies report that 13% consumed this beverage weekly or more, and 66% consumed at least once during the past year [23]. Other authors postulate that in United States, 25% of high school seniors, 34% of college students, and 34% of young adults consumed energy drink mixed with alcohol at least once during the past year [24, 25].

Due to the high consume of energy drinks associated or not with alcohol, the aim of this study was to evaluate the effects of using alcohol, energy drink and the mixture of these two beverages in the metabolic profile and behavioral parameters of Wistar rats.

2. Methods

2.1. Ethics

This experiment had the approval by the Animal Research Ethics Committee of the University of Marília (UNIMAR, Marília, SP, Brazil).

2.2. Group of animals

Forty male Wistar rats with 100g to 120g were part of the experiment. They were maintained in the vivarium at the Medical School of Marilia – UNIMAR (CEMA). Animals were housed in collective cages under a dark/light cycle of 12 hours, room temperature of 22 ± 2°C, and relative air humidity of 60 ± 5%. Animals were fed and watered *ad libitum*; *and* cared according to the recommendations of the Canadian Council's "Guide for the care and use of experimental animals".

Rats were divided in 4 groups (n=10), acclimated to laboratory conditions (for seven days), and treated according to the following groups (all the animals were fed with regular rat feed *ad libitum*):

G1: Control group that was fed and watered *ad libitum;*

G2: Group treated with *cachaça* (sugar cane spirit that contain 40^0 of alcohol) mixed with water (1:1) *ad libitum.*

G3: Group treated with energy drink (energy drink and water 1:1) *ad libitum*;

G4: Group treated with *cachaça* and energy drink (1:1) *ad libitum*.

2.2. Metabolic parameters

After a period of 45 days, the rats were euthanized with a lethal intraperitoneal injection of 200 mg/Kg of thiopental. After death, blood samples were obtained from the vena cava to determine the biochemical parameters: total cholesterol, high density lipoprotein (HDL-c), triglycerides, glycaemia, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatine phosphokinase (CPK). The glucose and lipid levels were measured in mg/dL; AST, ALT and CPK in U/L.

Atherogenic Coefficient (AC), Atherogenic Index (AI), Cardiac Risk Ratio 1 (CRR1), and non-HDL-c levels were studied following Ahmadvand *et al.* [26], Erejuwa *et al.* [27], and Ikewuchi *et al.* [28]: AC = (Total cholesterol – HDL-c)/HDL-c; AI = log (Triglycerides/HDL-c); CCR1 = Total cholesterol/HDL-c; CCR2 = LDL-c/HDL-c; Non-HDL-c = Total cholesterol - HDL-c.

2.3. Behavioral testing

In order to perform the behavioral test, animals underwent the Elevated Plus Maze to evaluate the anxiety index according to the model of Boerngen-Lacerda *et al.* [29] and Blanchard *et al.* [30].

This apparatus is constructed with wood and possess two open and opposed arms (50x10 cm), two enclosed arms (50 x 10 x 40 cm) and platforms with the same extent of the open arms, and that cross them perpendicularly, delimiting a central area of 10 cm^2. The apparatus stands 50 cm high from the ground and the rats are placed in for 5 minutes. This procedure was performed at the end of the experimental protocol (45 days of treatment). Parameters as time and frequency in the open and close arm, and in the center; time and frequency of lifting, strengthening, diving and self-cleaning were analyzed.

2.4. Statistical analysis

T-Test and ANOVA were performed for the statistical analysis and the variables were presented as mean and standard error mean, adopting a 5% level of significance.

3. Results

Our results show that food consumption decreased significantly in the groups treated with alcohol and energy drink but did not vary in the group treated with the mix of both beverages. Body weight at the beginning and at the end of the experimental protocol is not different when comparing the groups (Table 1).

Table 1: Mean and standard deviation of the food comsumption and body weight of G1: Control Group; G2: Group treated with alcohol; G3: Group treated with energy drink; G4: Group treated with alcohol mixed with energy drink.

Parameters	G1	G2	G3	G4	p-value
FC	114.4±9.53	82.44±17.65	68.13±19.36	107.53±8.91	0.000*
BW1	159.35±19.57	164.55±16.05	165.35±17.35	155.8±18.60	0.600
BW2	324.6±28.52	310.7±29.19	312.15±12.89	331.7±26.64	0.670

FC: Food consumption (mL/kg); BW1: Body weight at the beginning of the treatment (g); BW2: Body weight at the end of the treatment (g). *Level of significance: 5%.

In table 2 it is possible to observe that the consumption of the beverages did not modify significantly the values of glycaemia, HDL-c, AST, ALT and CPK but promoted significant increase in the levels of triglycerides and total cholesterol.

Table 2: Mean and standard deviation of the biochemical parameters of G1: Control Group; G2: Group treated with alcohol; G3: Group treated with energy drink; G4: Group treated with alcohol mixed with energy drink.

Parameters	G1	G2	G3	G4	p-value
Glycaemia	213.6±82.46	207.9±100.78	183.5±84.8	165.3±45.42	0.520
TG	72.5±20.01	113.7±36.81	97.8±26.67	89.7±39.44	0.041*
TC	40.6±4.13	48.9±1.86	47±3.52	51.5±2.23	0.002*
HDL-c	19.9±2.42	19.4±2.11	21.6±2.06	20.6±2.41	0.171
AST	119.3±31.67	106.1±15.76	121.8±70.43	83±9.67	0.130
ALT	69.9±11.26	54.4±5.6	56.9±16.65	53±8.99	0.077
CPK	1222±795.17	1194.6±647.7	958.5±382.7	788.2±442.61	0.311

TG: triglycerides; TC: total cholesterol; HDL-c: High Density Lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CPK: creatine phosphokinase.

*Level of significance: 5%.

Results for atherogenic indices and non-HDL-c values are found in table 3. Significant increase is seen only for AI.

Table 3: Mean of the Atherogenic Indices and non-HDL-c of G1: Control Group; G2: Group treated with alcohol; G3: Group treated with energy drink; G4: Group treated with alcohol mixed with energy drink.

Parameters	G1	G2	G3	G4
AC	1.35	1.25	1.26	1.47
AI	0.51	0.78*	0.65*	0.68*
CRR1	2.11	2.16	2.14	2.39
Non-HDL-c	26	24	25	28

AI: Atherogenic Index; AC: Atherogenic coefficient; CRR1: Cardiac Risk Ratio 1. *P<0.01, according to Dunnett Test.

Results for the behavioral test (Table 4) show that the use of the beverages significantly modify the frequency in the open and closed arm and in the center. They also decrease the time in the center of the Elevated Plus Maze.

Table 4: Mean and standard deviation of the Behavioral testing of G1: Control Group; G2: Group treated with alcohol; G3: Group treated with energy drink; G4: Group treated with alcohol mixed with energy drink.

Parameter	G1	G2	G3	G4	p-value
Time in the open arm (s)	85.7±54.8	84.4±27.8	98.3±42.5	50.7±37.2	0.086
Frequency in the open arm	3.9±2.3	5.9±3.1	6.7±2.6	6.1±1.8	0.009*
Time in the close arm (s)	129.5±66.5	169.1±37.5	140.0±51.9	186.4±66.1	0.390
Frequency in the close arm	4.9±2.7	6.2±3.1	8.6±3.8	5.4±3.2	0.007*
Time in the center (s)	84.7±76.3	46.5±19.3	61.6±16.6	62.8±39.5	0.009*
Frequency in the center	8.0±1.6	11.1±5.4	15.2±5.8	9.1±4.7	0.007*
Time of lifting (s)	2.40±1.2	2.44±0.92	3.1±0.9	1.87±0.9	0.160
Frequency of lifting	13.1±6.4	14.7±5.7	17.0±5.45	10.5±5.19	0.090
Time of strenghing (s)	1.32±0.7	1.4±0.8	1.2±0.8	1.1±0.9	0.680
Frequency of strenghing	6.8±3.4	8±4.4	6.4±4.4	5.9±5.2	0.740
Time of diving (s)	1.1±0.6	1.2±0.9	1.3±0.7	0.6±0.5	0.250
Frequency of diving	5.8±3.6	7.7±5.2	6.9±3.8	3.2±2.30	0.068
Time of self-cleaning (s)	0.9±0.8	0.8±0.6	0.6±0.6	0.7±0.6	0.360
Frequency of self-cleaning	4±3.4	3.6±2.6	2.6±2.4	2.7±2.6	0.63

*P<0.01, according to Tukey Test.

4. Discussion

The observation of our results regarding body parameters, glycaemia, HDL-c, AST, ALT, CPK, AC, CCR1 and non-HDL-c revealed that in the forty five experimental days, the animals fed with alcohol, energy drink and the mix of these beverages did not present significant diferences when compared with the control group. Significant increase were found for triglycerides and total cholesterol levels indicating that these drinks may affect these parameters. We also observed reduction on the food intake in the groups treated with alcohol and with energy drinks but not with the mix of these beverages.

Energy drinks are usually consumed mixed with alcohol possibly to diminish the sedative effects of alcohol or increasing the duration of an alcohol drinking session but energy drink alone may also represent an important part of the overall picture once this mixture may also facilitate the ingestion of higher amounts of alcohol [17, 23, 31].

Ebuehi *et al.* [16] studied the effects of energy drinks in rabbits and found no differences in the body weight and feed intake but found increase in CPK, AST and ALT levels. They also found increase in the levels of blood glucose, total cholesterol, triglycerides, HDL-c LDL-c. They also found significantly higher values of brain acetylcholine indicating that the use of this kind of beverage may alter cholinergic neurotransmission and neural functions mediated by acetylcholine.

Energy drinks possess a natural methylopxanthine named caffeine that is a psychoactive substance capable of blocking A1 and A2 adenosine receptors and in high doses inhibits the phosphodiesterase activity. It may decreases calcium ion accumulation in the mitochondria of cardiomyocytes and may increase blood pressure, arterial wall stiffness, and endothelium-dependent flow mediated dilatation. Caffeine also may elevate total cholesterol levels and high consume are related with acceleration of acute ischemic cardiac disease [32].

Energy drinks may increase metabolic risk also due to the presence of high content of sugars as fructose that may lead to weight gain, increase in the levels of blood glucose, total cholesterol, triglycerides and decrease of HDL-c levels. Authors have shown that the intake of sugar-sweetened beverages as high sweetness of diet may result in conditioning for a greater preference for intaking sweetened items. Furthermore, components in the beverages may induce insulin resistance, oxidative stress and pro-inflammatory conditions that are related with development of diabetes, metabolic syndrome and cardiovascular diseases [33, 35].

Previous studies have also shown relationship between alcohol consumption and the increased levels of blood lipids such as levels of total cholesterol and triglycerides [36, 37].

The increase of total cholesterol, triglycerides and AI in G2, G3 and G4 indicate that consume of alcohol, energy drink and both mixed may augment the risk of oxidative stress and inflammation [39]. These conditions are known to predict higher risk of cardiovascular diseases that are among the main causes of death in the modern world. The risk factors for formation of atherogenic plaques besides due to other factors, are also related to hypertriglyceridemia, and high levels of cholesterol. Atherosclerotic lesions are caused by a series of specific cellular and molecular responses normally related to oxidation and inflammation. Alcohol consumption as well energy drinks may be related to increase of lipids levels and induce to oxidative stress and inflammation [40, 43].

Similarly to our results, literature provides several evidences on risks associated with consumption of energy drinks and alcohol and suggest that consumers are at higher risk of cardiovascular diseases, and other problems as diabetes and metabolic syndrome [44, 45].

Our results showed that some parameters in the behavioral test (Table 3) were altered in the groups that consumed only alcohol, energy drink or the mixture of both. Holubcikova *et al.* [46] studied 8502 adolescents regarding the use of alcohol and energy drinks and found that adolescents consuming both alcohol and energy drinks were at higher risk of negative behavioural outcomes such as bullying, school dislike, truancy, fighting and low academic achievement, when comparing to those who were no consumers or that drank only alcohol or energy drinks. Other authors performed similar study and found similar results [47, 48]. The negative behavior may be associated with high consume of alcohol when it is mixed to a sweet and nice flavor beverage as energy drinks. This may facilitate the high ingestion, induces the decrease of sleepiness and extends the sedating effects what may also extend the drinking session leading to impairs judgement and neurocognitive functioning. Alcohol and specific compounds of the energy drinks such as caffeine and taurine may contribute to an aggressive behavior [46].

5. Conclusion

Based on our results we may say that the consumption of alcohol, energy drinks and the association of these beverages may increase risk for cardiovascular diseases once they increase Atherogenic Index and levels of cholesterol and triglycerides. Furthermore, they may also lead to behavioral outcomes.

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