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THE IMPACT OF 5- HOUR ENERGY® DRINK ON QTc AND HEART RATE PARAMETERS

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The research work was carried out at David Grant USAF Medical Center, Travis AFB and University of Pacific, Stockton under the supervision of my mentors Prof. Aleš Mrhar, PhD, PharmD and Associate Professor Sachin Shah, PharmD.

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STATEMENT

I declare that I have carried out my diploma work independently under the mentorship of Prof. Aleš Mrhar, PhD, PharmD and Assoc. Prof. Sachin Shah, PharmD.

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ABSTRACT

Energy drinks represent one of the most dynamic segments of the soft drinks market. Young people (students, teenagers) are the most common consumers of energy drinks driven by a strong belief that they are a good source of instant energy.

Due to lack of knowledge about energy drinks, we have low awareness about their effects on human body, especially the heart. The safety of energy drinks is therefore quite unknown. The most common ingredients are caffeine, vitamins B, taurine, and sugar.

Energy shots can be considered as energy drink concentrates, due to similarity in their chemical components. The 5-Hour ENERGY® drink is one of the top selling energy shot brands in the US. While cardiovascular diseases are the number one leading cause of death today, the aim of our study was to assess the cardiac effects of a 5-Hour ENERGY® drink on electrocardiographic parameters of healthy subjects, especially the QT interval and heart rate.

According to the FDA criteria, all drugs whose therapeutic dose causes QT/QTc for prolongation >10ms, need intensive electrocardiographic assessment in further procedure.

We set up a randomized, double blind, placebo-controlled, crossover study design for evaluation of cardiovascular effects of the 5-Hour ENERGY® drink. The participants were randomized into two groups. They received either placebo or the energy drink (7 days phase I, washout, 7 days phase II). The study included 18 healthy subjects aged from 18 to 40 years. In this study the maximum recommended dose of 5-Hour ENERGY® drink (two bottles a day spaced out) were consumed by each participant.

We did not find significant differences between QT/ QTc intervals of healthy subjects after drinking a single dose of 5-Hour ENERGY® drink and seven days after its chronical consumption. Because the QT is dependent on the heart rate, meaning the faster the heart rate, the shorter the QT interval, we corrected all measured values by using Bazett's and Friederica formulas. Three hours after drinking a single dose of 5-Hour ENERGY® drink, statistically significant change in heart rates was detected.

With our study, we cannot conclude that 5-Hour ENERGY® drink is safe; although we have established that it does not cause statistically and clinically significant differences on QTc if consumed within daily recommended quantities as indicated on the 5-Hour ENERGY® drink bottle.

Keywords:energy drinks, 5-Hour ENERGY® drink, QT interval, heart rate, ECG

RAZŠIRJENI POVZETEK

Energijske pijače predstavljajo enega izmed najhitreje rastočih segmentov trga brezalkoholnih pijač. Športniki, najstniki in študenti so njihovi najpogostejši potrošniki. Zaradi pomanjkanja znanja o delovanju energijskih pijač imamo ljudje nizko ozaveščenost o njihovem vplivu na človeško telo, še posebej na srce. Njihova varnost je še vedno slabo raziskana in zato neznana. Najpogostejše sestavine energijskih pijač so kofein, vitamin B, taurin in sladkor.

Energijski „shot“ vsebuje enako količino kofeina kot navadna pločevinka energijske pijače (360–470 ml), le da je skoncentriran 60 ml.

5-Hour ENERGY® drink je eden izmed najbolj prodajanih energijskih „shotov“ v Združenih državah Amerike.

Kardiovaskularne bolezni so danes eden od najpogostejših vzrokov smrti. Cilj študije je bil oceniti vpliv 5-Hour ENERGY® drink-a na elektrokardiografske parametre zdravih oseb, še posebej na QT-interval in srčni utrip.

V skladu z merili FDA potrebujejo vsa zdravila, katerih terapevtski odmerek povzroči podaljšanje intervala QT/QTc > 10 ms, intenzivno oceno elektrokardiografskih parametrov v nadaljnjem postopku.

Izvedli smo randomizirano, dvojno slepo, s placebom nadzorovano, navzkrižno študijo za ocenjevanje kardiovaskularnih učinkov 5-Hour ENERGY® drink- a.

Udeleženci so bili naključno razporejeni v dve skupini; prejeli so bodisi placebo ali energijsko pijačo (7 dni faza I, izpiranje, 7 dni faza II).

V raziskavi je sodelovalo 18 zdravih oseb, starih od 18–40 let.

V študiji smo uporabili največji dnevno priporočeni odmerek 5-Hour ENERGY® drink- a, to je dve steklenički na dan.

V raziskavi nismo ugotovili statistično značilnih in klinično pomembnih razlik QT/ QTc zdravih oseb tako po zaužitju enkratnega odmerka kot tudi po 7-dnevnem uživanju 5-Hour ENERGY® drink- a. Ker je vrednost QT-intervala odvisna od srčnega utripa, pričemer pomeni višji srčni utrip krajši interval QT, smo popravili vse izmerjene vrednosti z uporabo formul Bazett in Friederica. Tri ure po zaužitju enkratnega odmerka 5-Hour ENERGY® drink-a pa smo opazili statistično značilne spremembe srčnega utripa.

Na osnovi rezultatov raziskave ne moremo zatrditi, da je uživanje 5-Hour ENERGY® drink-a varno. Ugotovili smo le, da ne povzroča statistično in klinično značilnih sprememb QTc, če ga zdravi ljudje uporabljajo v dnevno priporočenih odmerkih, ki so navedeni na zunanji ovojnini.

Ključne besede: energijske pijače, 5-Hour ENERGY® drink, QT-interval, srčni utrip, EKG

LIST OF ABBREVATIONS

BP- blood pressure

CNS- central nervous system

CVD- cardiovascular diseases

DBP- diastolic blood pressure

ECG- electrocardiograph

EDs- energy drinks

GI- gastrointestinal tract

HR- heart rate

OTC- over the counter drugs

SBP- systolic blood pressure

SD- sudden death

TdP- Torsades de Pointes

1. INTRODUCTION

Energy drinks (EDs) represent one of the most dynamic segments of the soft drinks market, reaching a sale of 4.8 billion liters in 2011. In United States, 8.6 billion dollars were spent on EDs in 2012 (1). The EDs market is continuously growing worldwide, while the consumption of other sugar beverages is globally decreasing (2). Young people (students, teenagers) are among the most common consumers of EDs, driven by a strong belief that they are a good source of instant energy. Unfortunately the vast majority is unaware of their harmful effects on our health.

The price of EDs is usually two times higher than the price of regular soft drinks. Their popularity amongst teenage populations could be explained by easy accessibility of the same in local shops, big grocery stores, gas stations, and clubs. There is a whole range of different EDs available on the market, with a bunch of new flavors such as coffee/tea and various fruits. They are mostly marked as wake-uppers in cans or as a shot giving you a lot of energy.

EDs are usually sold in non-resealable cans that you have to drink in one time. Their volume varies from 8 oz. (236 ml) to 24 oz. (710ml) cans (3).

Due to the lack of knowledge about the effects of EDs, we have low awareness about effects of EDs to human body, especially the heart.

The most common ingredients are unhealthy levels of caffeine, vitamins B, taurine, and sugar. Other commonly used additives can include sodium, folic acid, guarana, carbonated water, yerba mate, various forms of ginseng, maltodextrin, inositol, carnitine, creatine, glucuronolactone, sodium benzoate, ginkgo biloba, purified water, natural and artificial flavors, etc (4).

1.1. ENERGY DRINK INGREDIENTS

Caffeine

Caffeine can be isolated from more than 60 plants species, including coffee beans, tea leaves, kola nuts, guarana, yoco, and mate (5). Soft drinks, coffee and tea are some of the caffeine sources.

The caffeine absorbtion is through GI tract (99%). Within 50 to 75 minutes it reach peak plasma level. It is eliminated by first-order kinetics. Caffeine half- life in healthy adults is around 6 hours (6).

Caffeine causes the release of noradrenaline by increasing calcium intracellular stores in the cells. However, cardiovascular events such as ventricular tachyarrhythmias or supraventricular tachyarrhythmias may be result of caffeine effects to dopamine receptors, particularly when the doses of caffeine are high (6).

Caffeine is absorbed by all body tissues, and it can easily cross the blood-brain and placental barriers (7, 8).

For therapeutic purposes, caffeine is widely used in combination with prescription drugs and over-the- counter (OTC) medications. It is an analgesic additive in combination with acetylsalicylic acid, or with paracetamol and acetylsalicylic acid, in which case it enhances pain relief (9).

In combination with ergotamine caffeine is used for treatment migraine headaches (10).

Caffeine content and concentrations vary widely between different EDs. The advised daily intake is no more than 400 mg. Possible lethal dose is 3 g of caffeine (11). From 95-200 mg of caffeine is contained in an ordinary cup of coffee. For therapeutic purposes, doses for caffeine are from 250 mg (for headache) to 300 mg (for headache after epidural anesthesia). Some EDs contain from 50- 300 mg of caffeine per serving (12).

Taurine

Taurine is a non-essential sulfur-containing amino acid found in animal tissue, but it is not amino acid in the usual meaning because of its component of protein (13). It was first identified and isolated from the bile of the ox (*Bostaurus*), from which it derives its name (14).

Taurine is involved in the osmoregulation, reproduction, immunity, membrane stabilization and development of the central nervous system (15). Taurine's chemical structure shows that it contains sulphonate groups, but not the carboxyl that is typical for other amino acids. In dietary sources, taurine is found in food, especially in seafood and meat. In clinical practice it is for the treatment of diabetes, hepatitis, alcohol withdrawal, cystic fibrosis, seizure disorders (16).

The European Food Safety Authority reports that taurine doses up to 1000 mg/kg per day do not have adverse effects. The recommended dosage levels of taurine are up to 5 grams daily. Amounts of taurine in EDs vary between 600 to 1000 mg. It is thought that taurine increase the caffeine effects. So far, no documentations supporting adverse effects caused by taurine has been recorded, however due to few death cases in Europe, it has been attached to the drinks containing taurine and caffeine. A limited or banned sale of EDs with taurine is in some countries (17, 65).

Glucose

Glucose is a main source of energy for the cells, brain and muscles. It is an essential component for the normal functioning of the CNS.

EDs contains approximately 27g of glucose per 8-oz serving. U.S. guidelines currently recommend a maximum sugar intake of 32g for every 2000 calories. EDs in 16- or 24-ounce sizes contains 60–90g of glucose (17).

Effects of the feeling of sugar high occur from an increase in blood sugar levels and give a temporary boost in energy. High dose of sugar is followed by a break down. It is also known as a “crash mode”, a state in which the body perceives more sugar is needed in order to function normally (18). A lot of diseases such as diabetes, low serotonin levels, oral dental erosions or cavities, obesity and upsetting the balance of healthy bacteria in the GI tract may be caused by high sugar diet (17, 19).

Niacin

Niacin is an organic compound also known as vitamin B3. Recommended daily doses are 30mg for adults (20). It can be found in meat, green vegetables, fish, beans, milk, cereal grains and eggs. Niacinamide, another form of vitamine B3, is different from the niacin. In

niacinamide, the carboxyl group is replaced by a carboxamide group. Both are found in many vitamin B complex supplements, along with other B vitamins.

As vitamin B3, niacinamide and niacin are used for preventing pellagra. Other therapeutic usages are for high cholesterol, migraine headache and dizziness. Some people are using niacin or niacinamide for improving circulation, memory loss, promoting relaxation and acne (21).

For most people, niacin is likely safe when taken orally. Flushing reaction is one of the common side effects of niacin. Doses of over 3g per day of niacin may cause more serious side effects. A typical 8 fl-oz EDs serving contains approximately 20mg of Niacin, which is close to the recommended daily doses. For example, one bottle of 5-Hour ENERGY® drink serving size 1.93 fl-oz contains 30mg, while a bottle of 24 fl-oz Monster contains 120mg of niacin (22, 23).

Folic acid

Folic acid is a water-soluble vitamin B9 which is essential for many important body functions. Our bodies cannot synthesize it; therefore, it has to be supplied through the diet to meet our daily requirements. Foods that are rich with folic acid are leafy, beans, meat, orange juice, meat, mushrooms. It is used for preventing and treating low blood levels of folic acid (folic acid deficiency. Used during the preconception period, it helps protect against some birth defects (24).

Recommended amount of every-day consumption is 400mcg (micrograms). One serving of 5-Hour ENERGY® drink contains 400mcg of folic acid. Some adverse effects induced high doses of folic acid are nausea, skin reaction, stomach upset and sleep disorders (24).

Vitamin B6

Vitamin B6 is, as pyridoxine, part of vitamin B complex. It acts as a coenzyme to many other enzymes and it is involved in many metabolic processes. It can be found in eggs, meat, vegetables, liver and cereals. Vitamin B6 is used for treating sideroblastic anemya, pyridoxine deficiency, some type of seizures, reducing high blood level of homocysteine, for upset stomach and vomiting in pregnancy, kidney stones, breast pain and depression as premenstrual syndrome symptoms, boosting the immune system, and Alzheimer's disease. Taken at the

recommended dose, it is likely safe for most people. Some of the side effects caused by vitamin B6 are vomiting, loss of appetite, headache, nausea, stomach pain, and sleepiness, brain problems (25).
The upper safe limit for adults is 100mg. Content of niacin in 5- Hour Energy® drink is 40mg (25).

Vitamin B12

Vitamin B12 is also called cobalamin and it is important for a normal functioning and development of brain, red blood cells and nerves. It can be found in milk, milk products, eggs, meat and fish. In therapeutic purposes, it is used for treatment of vitamin B12 deficiency, pernicious anemya, improving thinking and memory in persons aged 65 and older, and treatment of age-related macular degeneration. Recommended dietary allowance is 2.4mcg per day, and the content of cobalamin in 5-Hour ENERGY® drink is 500mcg, which is 208-times higher than the recommended daily dosage (26).

Riboflavin

Riboflavin is known as vitamin B2 and it has a key role in human health. It can be found in meat, leaf vegetables, liver, yeast, mushrooms, almonds, kidneys, and cheese (27).
It is used for treating congenital methemoglobinemia, riboflavin deficiency, carpal tunnel syndrome and cervical cancer. Recommended daily intake is 1.3mg. Content of riboflavin in some EDs is 1.7mg per serving (27).

Guarana

Guarana is a plant, native to the Amazon basin and especially to Brazil. As a dietary supplement, it is an effective stimulant. Extracts of seeds of the fruit of guarana contain caffeine at concentrations that are three times higher than their concentration in coffee beans. In the amount of 3–5g, guarana provides 250 mg of caffeine. This substance is generally regarded as safe (GRAS) by the FDA, when not combined with other stimulatory agents, such as ephedra and amphetamines (28).

Ginseng

Ginseng is another herbal supplement, common to many EDs. It is not approved for use in beverages by FDA and it does not have GRAS status on FDS's list. It is FDA-approved as a food additive for flavor (29). It is a dietary supplement whose mechanism of action is still unknown. Panax ginseng is possibly effective for diabetes, thinking and memory, and chronic obstructive pulmonary disease. It is possibly safe when it is not used for longer than three months and if it is taken orally. Side effects are increased heart rate, loss of appetite, insomnia, mood changes, diarrhea, itching, and rashes. It is not recommended taking Panax ginseng with caffeine. It interacts with some medications for depression, diabetes, anticoagulants, and Warfarin. EDs contains around 200 mg per serving, while the recommended daily dosage is between 1 and 2 grams (4).

1.2. ENERGY DRINK VS ENERGY SHOTS

In case you are wondering what is the difference between caffeinated drinks and energy shots, there is no clear rules that would define the difference between the EDs and an ordinarily caffeinated drink such as coffee or soda have been set yet. But generally speaking, EDs contains 3 times more caffeine than an average can of cola.

Energy shots can be understood as energy drink concentrates, due to similarity in its chemical components.

1.3. 5- HOUR ENERGY® DRINK

One of the top selling energy shots brands in the US is 5-Hour ENERGY® drink. In 2012, they sold for 896 million US dollars of the beverage (1).

5-Hour ENERGY® drink has been marked as a potent energy “shot” that gives the boost of regular coffee or EDs, but without the “crash” in energy later. Their slogan is “hours of energy now, no 2:30 feeling later”, which alludes to their claim of boost in energy without a crash in energy later, which is typically experienced with other EDs (30).

The 5-Hour ENERGY® drink label claims to include a blend of Niacin (30mg), Vitamin B6 (40mg), Folic Acid (400mcg), Vitamin B12 (500mcg), Sodium (18mg), and an Energy Blend consisting of Taurine, Glucuronolactone, Malic Acid, N-Acetyl L-Tyrosine, L-Phenylalanine, Caffeine, Citicoline (1870mg). Other ingredients are purified water, natural and artificial flavors, sucralose, potassium sorbate, sodium benzoate, and EDTA (30).

Poisoning with EDs and caffeine toxicity have been burning topics. Caffeine has been reported and shown to have an effect on blood pressure, but not with an associated QTc prolongation or influence on RR interval (31, 32).

Abnormal heart rhythms and heart palpitations have been reported with IV administration of niacin (21). The rest of the ingredients of 5-Hour ENERGY® drink may contribute to a change in blood pressure or a change in heart function, but their exact interaction with each other is not clearly understood (33).

Other EDs have been evaluated for their safety, but their trials were not well controlled, the dosing not practical, underpowered, or were short in duration (32, 34).

However, the safety of energy drinks is unknown.

1.4. ECG PARAMETERS AND BLOOD PRESSURE

Cardiovascular diseases are the number one leading cause of death today. Some of the factors that contribute to the development of cardiovascular diseases (CVD) are diabetes, dyslipidemia, hypertensions, smoking, drinking too many caffeinated beverages, and an unhealthy way of living.

Heart rate is the most basic timing measurement of electrocardiograph (ECG). Heart rate is defined as how many times a heart contracts and relaxes per minute. Slower heart rate will be associated with longer distance between QRS complexes (35).

The QT interval is the time between the depolarization of the first ventricular cell and the repolarization of the last ventricular cell. It provides a rough estimate of the duration of the plateau phase of the ventricular tissue (36, 37).

The QT interval is influenced by heart rate, age and gender. When heart beats slower it is longer and when heart beats faster the QT interval is shorter. Because of that it is necessary to correct QT interval value by using mathematical formula.
Fridericia's cube root formula ($QTc = QT/\sqrt[3]{RR}$) and Bazett's square root formula ($QTc= QT/\sqrt{RR}$) are two most commonly used formulas. RR is length of interval two R waves.
QTc interval greater than 440ms is considered for QT prolongation (39). The QTc interval is longer in women because of a relative shortening of the QTc interval in men during adolescence (38). QTc interval considered as normal in women is less than 440 ms and less than 420 milliseconds.

Sometime using term QT or QTc prolongation can be confusing. Also in many scientific articles there is no unique way of using one or the other term. As well in many literatures there is frequently use as reference value QTc.
But usually when we are talking generally about prolongation we will use the term "QT prolongation". When we want to consider the influence of heart rate to QT, we will correct it and use term QTc.
Until the most studies are only calculating QTc, in our study we calculated both datas because we want to see if our results change with heart rate.
In other words, prolongation of QT or prolongation of QTc is not good.

Causes for QT prolongation are congenital and acquired. Among congenital causes are Romano-Ward syndrome and Jervell and Lange-Nielsen syndrome, while the acquired ones are ischemia, heart failure, bradycardia, hyperthyroidism/hypothyroidism, hypokalaemia, hypomagnesaemia and drugs. Many drugs can cause prolonged QT interval (41). It is assumed that the mechanism of drug-induced QT prolongation is the blockage of cardiac potassium channels. The prolongation caused by drugs is usually seen within several days of taking it.
QTc prolongation is linked with sudden cardiac death and Torsades de Pointes (TdP).
Some drugs associated with QT prolongation are antiarrhythmics, antipsychotics, antihistamines, antidepressants, antifungals, antibiotics, antivirals, antimalarials, and anaesthetics (42).

Before prescribing, doctors should be familiar with drugs that may cause QT prolongation; they also have to be very careful when prescribing these drugs to patients who are already taking some other drugs. Co-administration of many drugs should be avoided. It is of a big importance to question a patient about the usage of non-prescribed medications. Apart from drugs, there is also a possibility of increasing the risk of the drug-induced QT prolongation with grapefruit juice, which is inhibiting the metabolism of amiodarone (antiarrhythmics) (43).

According to the FDA criteria, all drugs whose therapeutic dose causes QT/QTc prolongation for > 10ms need intensive ECG assessment in further procedure. The FDA guidance states the following: The threshold level of regulatory concern, discussed further below, is around 5ms, as evidenced by an upper bound of the 95% confidence interval around the mean effect on QTc of 10ms. But "positive" does not mean strictly that the drug is proarrhythmic. Some adverse events could also increase proarrhytmic risks (44).
The Thorough QT/ QTc study (TQT) is a clinical pharmacology study which assesses whether a drug has a threshold pharmacological effect on cardiac repolarization, as detected by QTc prolongation (44).
Except QT interval, there are also other intervals that could be measured from the ECG.
The PQ interval shows how fast is the transmission of the action potential through the atrioventricular (AV) node from the atria to the ventricles. The PQ interval considered as normal is from 0.12 and 0.20 seconds. Sometimes, the PQ interval is termed PR interval when the Q wave is not present.
The QRS interval indicates the speed of the ventricular depolarization. The normal QRS is <0.10 seconds (40).

Torsades de Pointes is a polymorphic ventricular tachycardia. It is characterized by twisting of the QRS complexes around the isoelectric line. It is caused by prolonged or abnormal repolarization of the ventricular myocardium. Repolarization is the return of the ions to their previous resting state. Myocardial muscle is in resting state and is preparing for a new phase. (45)

TdP is associated with prolonged QT interval or abnormal T or U/T morphology.

The general risk factors of TdP are heart disease, bradyarrhythmias, female gender, electrolyte abnormalities, stroke, and a significant universe of drugs that lead to QT prolongation (46).

For the treatment, the offending agent has to be withdrawn. Additional treatment is giving the infusion of magnesium sulfate, isoprenaline infusion or the electrical defibrillation.

An important factor of cardiovascular events is blood pressure (BP) (47). Blood pressure is measured as systolic (SBP) and diastolic (DBP) pressures. SBP is the pressure when the heart beats while DBP is when the heart is at rest between beats (48). Normal value for SB is less than 120 mm Hg, until for DBS is less than 80 mm Hg (49, 50).

2. AIMS AND OBJECTIVES

2.1. THE AIM OF THE STUDY

The aim of this study was to assess the cardic effects of a 5-Hour ENERGY® drink on electrocardiographic (ECG) parameters of healthy subjects.

2.2. SPECIFIC OBJECTIVES

Objective 1: We hypothesize that there is no difference in the QTc of healthy subjects between drinking a single 5-Hour ENERGY®drink versus placebo.

Objective 2: We hypothesize that there is no difference in the QTc of healthy subjects between drinking 5-Hour ENERGY®drink versus placebo chronically for seven days.

Objective 3: We hypothesize that there is no difference in the heart rate of healthy subjects between drinking a single 5-Hour ENERGY®drink versus placebo.

Objective 4: We hypothesize that there is no difference in the heart rate of healthy subjects between drinking 5-Hour ENERGY®drink versus placebo chronically for seven days.

Objective 5: We hypothesize that there is no difference in the systolic blood pressure of healthy subjects between drinking a single 5-Hour ENERGY®drink versus placebo.

Objective 6: We hypothesize that there is no difference in the systolic blood pressure of healthy subjects between drinking 5-Hour ENERGY®drink versus placebo chronically for seven days.

Objective 7: We hypothesize that there is no difference in the diastolic blood pressure of healthy subjects between drinking a single 5-Hour ENERGY®drink versus placebo.

Objective 8: We hypothesize that there is no difference in the diastolic blood pressure of healthy subjects between drinking 5-Hour ENERGY®drink versus placebo chronically for seven days.

Objective 9: We hypothesize that the hand measurement of heart rate from the RR interval will be no different than the automatically measured heart rate

Objective 10: We hypothesize that the hand measurement of heart rate from the QTc interval will not be different from the automatically measured QTc interval

3. MATERIALS AND METHODS

3.1.Materials

This study was approved by the Institutional Review Board. Informed consent was obtained for all study participants. The study took place at the Department of Defense at David Grant USAF Medical Center.

3.1.1. Samples

The study intervention was a 2-oz 5-Hour ENERGY® drink that is readily available in supermarkets, or a placebo. None of the excipients in the placebo are known to cause blood pressure or ECG rhythm changes and are readily available food additives. All materials were purchased from a local supermarket and the lot numbers were recorded.

The 5-Hour ENERGY® drink and the placebo were packaged in a sterile environment by a pharmacy technician in identical 2-oz bottles and checked by another staff pharmacist. The active and placebo interventions were made one week in advance and were stored in a refrigerator. Containers for 5-Hour ENERGY® drink and placebo were matching (2-oz red translucent bottle), labeled and stored safely in a refrigerator, and it was available to the investigator for subject distribution.

Before preparing the placebo, we mixed 5 different combinations, asked a few participants to try them, and they told us what combination was the best. The goal was the matching taste and color.

The placebo was prepared with 24ml of filtered water, 15ml of reconstituted lime juice (readily available from supermarket Safeway), and 20ml of cherry flavoring (regularly used flavoring in compounding). After mixing all compounds in a 250ml Erlenmeyer, we put it in 2-oz red bottles that looked the same as the 5-hour ENERGY® drink bottles.

In the laboratory, we had standard conditions for temperature and pressure as a temperature of 20°C (293.15 K, 68°F) and an absolute pressure of 101.325 kPa (14.696 psi, 1 atm).

3.1.2. Electrocardiography

Electrocardiography is the recording of the heart's electrical impulses by way of sensors, called electrodes. For our study, we used 12- lead electrocardiograph (PageWriter Trim III, Philips Health-care, Andover, MA). It provides 12 different views of the heart's electrical activity. The more leads we have, the better is our chance of interpreting the heart's electrical activity.

We could divide the 12 leads to bipolar leads, augmented leads, and pericardial (chest) leads. Bipolar leads are Lead 1, 2 and 3. Augmented leads are aVR, aVL and aVF. They are called augmented leads because they generate small waveforms on the ECG paper. Both the bipolar and augmented leads are also called frontal leads.

Pericardial leads are located on the chest and their locations are: the fourth intercostal space and the right sternal border for V1, the fourth intercostal space and the left sternal border for V2, the fifth intercostal space and the mid-clavicular line for V4, the fifth intercostal space and the anterior axillary line for V5, and the fifth intercostal space and mid-axillary line for V6. V3 is located between V2 and V4.

The heart is sending the electrical impulses through the conduction system and the body. Electrodes which we apply on the skin pick up theses impulses and send them through lead wires to a cable into the ECG machine. There, an amplifier magnifies the signal and a galvanometer converses the electrical signal into the mechanical energy which is recorded on the ECG.

3.1.2.1.Control features

In our study, we used the chart speed 25mm/s, also known as "normal speed". It regulates the speed at which the paper prints out the information. In case we want to change the speed to 50 mm/s, it doubles the width of the waves and complexes.

As sensitivity controls that regulate the height of the complexes, we used 1, which is the normal setting. Changing it to 2 doubles the height of the complexes. The value of ½ shrinks it.

For a good performance of the ECG, it is very important to use it properly calibrated. This process is called standardization. Throwing 1 mill volt of electricity into the ECG machine should cause the stylus to print out a square wave of 10 mm in height on the ECG paper.

Other important factors are position control, frequency response and stylus heat control. During the ECG measurements, the participants were asked to breathe normally. They were examined in lying position, with the ECG electrodes placed on their thorax and extremities.

3.1.3. Blood pressure measurements

The blood pressure including SBP and DBP was measured using an automated calibrated machine (Masimo SET Vital Sign Monitor, Welch Allyn, Skaneateles Falls, NY) and the same machine was used for all data.

3.1.4. Participants

Twenty-two healthy male and female participants took part in the study. An inclusion criterion was that the healthy male and female subject should be at least 18 years old and in general good health. The upper limit age was 40 years, based on the Framingham 10-year Risk for Heart Disease calculation; age above 40 becomes positively associated with increased risk for heart disease.

Subjects didn't drink any ED 1 week prior and during the course of the study. They were also refrained from consuming caffeinated products 48 hours before the baseline evaluation and throughout the study duration. In case they did consume caffeine containing beverages, it would be noted but the subject wouldn't have been excluded from the study.

While this study was the first one to evaluate the cardiac effects of 5-Hour ENERGY® drink, similar studies have been conducted by Dr. Shah's research group for Aloe Vera, Co-enzyme-Q10 and Echinacea (51, 52).
At each visit (every day, twice a day, except on Saturday and Sundays), the subjects were asked if they are experiencing any adverse effects. In case of an emergency, they would be referred to the Emergency Room at the David Grant Medical Center.

Pregnancy was ruled out by urine dip stick test, mandatory for all female volunteers. The pregnancy test occurred on days 1 and 15 of the study.

Inclusion criteria:

- Healthy male and female (active duty military or DoD beneficiaries or Civilian government employees) subjects at least 18 years of age (max 40 years of age) and in general good health expressing interest in participating in the study will be evaluated and excluded if any of the following are present.

Exclusion criteria:

- rhythm other than normal sinus, history of atrial or ventricular arrhythmia, family history of premature sudden cardiac death, left ventricular hypertrophy, atherosclerosis, hypertension, palpitations, T-wave abnormalities, baseline corrected QT (QTc) interval greater than 440 milliseconds (ms)
- thyroid disease
- type 1 or 2 diabetes mellitus
- recurrent headaches
- depression
- any psychiatric condition or neurological disorder
- history of alcohol or drug abuse
- renal or hepatic dysfunction
- concurrent use of drugs potentially interacting with either 5-Hour ENERGY® drink or effecting electrocardiographic or hemodynamic parameters (some examples are anticoagulants, Monoamine-oxidase inhibitors, over the counter (OTC) medications containing pseudoephedrine or phenylephrine, or any dietary supplements)
- all non-English speaking subjects and those that don't understand the study or consent process can't be part of the study
- if the subject refuses to sign the informed consent document or HIPPA authorization, they would be excluded as well

- pregnant or lactating females will be excluded from participation with urine dipstick tests used to confirm pregnancy (pregnancy test performed before each phase)
- those who have consumed 5-Hour ENERGY® drink or any type of ED within one week prior to randomization can't be part of the study
- subjects less than 18 and above the age of 40 can't be part of the study

3.2. Methods

3.2.1. Study protocol

All subjects signed an informed consent document and HIPAA authorization (health insurance portability and accountability) prior to any screening procedures being performed for research purposes. All study procedures were conducted in consent with ethical standards for research in human subjects. We randomized subjects by using a computer model on website www.randomization.com. Day 1 for the study was always a Tuesday, Wednesday or a Thursday to ensure that the subject always meets with the investigator for evaluation days. All the cardiovascular tests were performed at baseline (BL).

- Day 0: Screening and evaluation for inclusion in the study

- Day 1: 0700-0900: Evaluation of subject's ECG. The subject got 2oz of either 5-Hour ENERGY® drink or placebo to drink it. After the first dose, the subject returned every other hour for ECG evaluation for a total of 5 hours (At 1, 3, and 5 hours after initial dose; each visit will take 20 minutes). In the afternoon (1400-1600) the subject returned to be given the second dose of the study drink (5 minutes). During this time we have not done any ECG evaluation.

- Day 2-4: The subject returned to receive 2oz of either 5-Hour ENERGY® drink or placebo twice every day (5 minutes each visit; between 0700-0900 and 1400-1600).

- Day 5-6: On Saturdays and Sunday, they didn't meet with the study investigator because we gave them 4 doses for consumption at home.

- Day 7: 0700-0900: Evaluation of subject's ECG. The subject drank 2oz of either 5-Hour ENERGY® drink or placebo. After the first dose, the subject returned every other hour for ECG evaluation for a total of 5 hours (a 1, 3, and 5 hours after initial dose; each visit will

take 20 minutes). In the afternoon (1400-1600) the subject returned to be given the second dose of the study drink (5 minutes). During this time we have not done any ECG evaluation.

- Days 8-14: Subjects was not seen the study administrator. This was a washout period.

- Day 15: 0700-0900: Evaluation of subject's ECG. The subject then drank 2oz of either 5-Hour ENERGY® drink or placebo which was the alternative of what they got in phase 1. After the first dose, the subject returned every other hour for ECG evaluation for a total of 5 hours (At 1, 3, and 5 hours after initial dose; each visit will take 20 minutes). In the afternoon (1400-1600) the subject returned to be given the second dose of the study drink (5 minutes). During this time we have not done any ECG evaluation.

- Day 16-18: The subject returned to receive 2oz of either 5-Hour ENERGY® drink or placebo which was the alternative of what they got in phase 1 twice every day (5 minutes each visit; between 0700-0900 and 1400-1600).

- Days 19-20: On Saturdays and Sunday, they didn't meet with the study investigator because we gave them 4 doses for consumption at home.

- Day 21: 0700-0900: Evaluation of subject's ECG. The subject then drank 2oz of either 5-Hour ENERGY® drink or placebo which was the alternative of what they got in phase 1. After the first dose, the subject returned every other hour for ECG evaluation for a total of 5 hours (At 1, 3, and 5 hours after initial dose; each visit will take 20 minutes). In the afternoon (1400-1600) the subject returned to be given the second dose of the study drink (5 minutes). During this time we did not perform any ECG evaluation.

Outcome Measures:

Primary Outcome:

- QTc interval

Secondary Outcome:

- QT interval
- Heart rate
- Office systolic blood pressure (SBP)

- Office diastolic blood pressure (DBP)

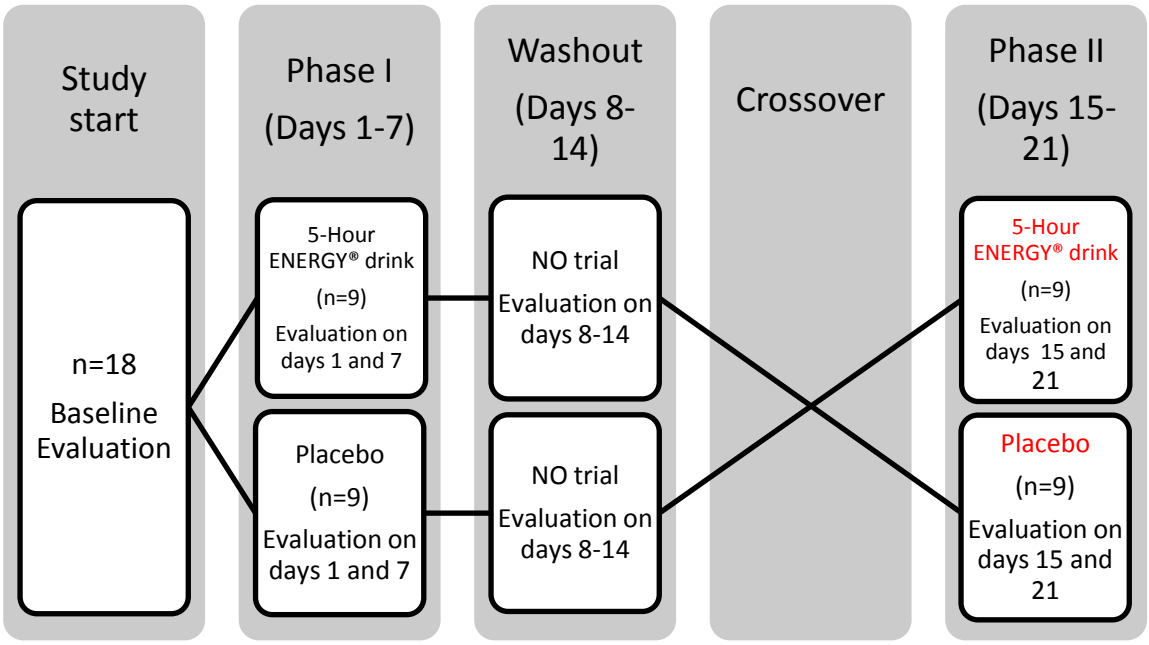

Table 1. *Study shema*

3.2.2. ECG hand measurement and interpretation of QT and RR interval

The QT and RR interval duration were compared between ECGs of 20 healthy subjects. The ECGs from two subjects were not useable because they dropped out.

We used a standard 12- lead ECG tracing at 25mm/s paper speed and 10mm/mV amplitude. Measurements of the ECG intervals were conducted manually, using the Adobe Acrobat Pro software. After we measured all ECGs, they were randomly verified by a third researcher.

RR and QT intervals were measured in lead II. The lead V5 was our alternative, in case that the lead II could not be read. For correct measurement, we first draw a line through the baseline, preferably through the PR segment. The QT interval extends from the beginning of the QRS complex to the end of the T wave. The easiest way to define Q wave is to find the first negative deflection from the baseline following the P wave. The end of the T wave was defined as the intersection of the tangent to the down slope of the T wave and the isoelectric

line. It is also very important to draw a tangent against the steepest part of the end of the T wave. If the T wave has two positive deflections, the taller deflection should be chosen. If the T wave is biphasic, the end of the taller deflection should be chosen. The heart rate was measured by using the distance between two consecutive R waves. The R wave is the first positive deflection following the Q wave. The QT interval was corrected according to Bazett's formula, in which the QT interval is adjusted for the heart rate by dividing it with a square root of the R-R interval (Q-Tc interval = Q-T interval ÷ √RR). For the heart rate, we used the following formula: HR = 1,500/RR interval in millimeters. **Table 2**. is showing a graphic representation of above mentioned intervals for better understanding .

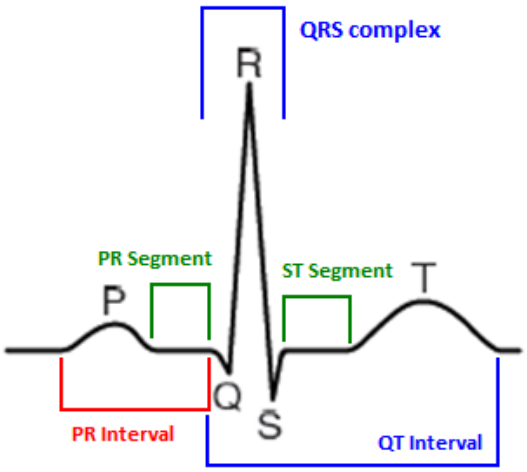

Table 2. *Electrocardiogram shema*

3.2.3. Statistical Analysis

This was a randomized, double blind, placebo-controlled, crossover study design evaluating the cardiovascular effects of 5-Hour ENERGY® drink. The participants were randomized into two groups; they received either the placebo or the 5-Hour ENERGY® drink.

The study included 18 healthy subjects aged from 18 to 40 years. The sample size was based to detect a difference of 10msec and a SD of 14msec (44). The calculated sample size by using paired t- test was 18 (75). We had normal distribution of the datas.

A p-value below 0.05 was considered significant with a power of 80%. As we mentioned in the study protocol, we were performing ECG on days 1, 7, 15 and 21 in intervals of 0 h, 1h, 3h and 5 hours after consuming 5-Hour ENERGY® drink or the placebo.

ECG intervals were analyzed as continuous variables. Statistical analysis was performed using Microsoft Office® Excel (2007). Demographic details were summarized for all randomized subjects using descriptive statistics.

Intergroup comparisons (placebo and 5-Hour ENERGY® drink groups will each comprise respective data from each subject, irrespective of the order of the studied drink administration) of continuous variables were performed using a student paired t-test.

All data are reported as mean ± S.D. A correlation coefficient will be calculated when necessary. A p-value of less than 0.05 was considered statistically significant. All electrocardiographic measurements were treated as continuous variables.

3.2.4. Adverse drug effects

It was also possible that the individual ingredients within 5-Hour ENERGY® drink may pose dermatologic side effects, such as erythema, urticaria, skin flushing, rash, itching, and rosacea fulminans (24, 24, 25). This study used the maximum recommended dose of 5-Hour ENERGY® drink (two bottles a day spaced out), so some of these side effects may be possible (30). The frequency of these effects currently remains unknown.

Adverse events were evaluated at all post-treatment time points and daily thereafter by asking participants if they had experienced any unpleasant feelings or symptoms since taking the experiment medication. Any symptoms reported were monitored for degree (mild, moderate, or severe), progression (same, worse, or improved), and resolution.

4. RESULTS

Twenty- two male and female subjects were enrolled into the study. Figure 1 shows the flow of participants throughout the study. Two of them did not meet the criteria for the study because of the inclusion/exclusion criteria. Both were using OTC medications containing pseudoephedrine or phenylephrine that could potentially either interact with the 5-Hour ENERGY® drink or could affect the electrocardiographic or hemodynamic parameters. Two other subjects dropped out during the study without explanation. In total, 18 subjects that met the criteria for analysis were included and completed the study, 5 of whom were female and 13 male. Thirteen were Caucasian, three were African American, one was Asian, and one was African American Pacific Islander. Patients were followed in various period of time depending on when they were enrolled.

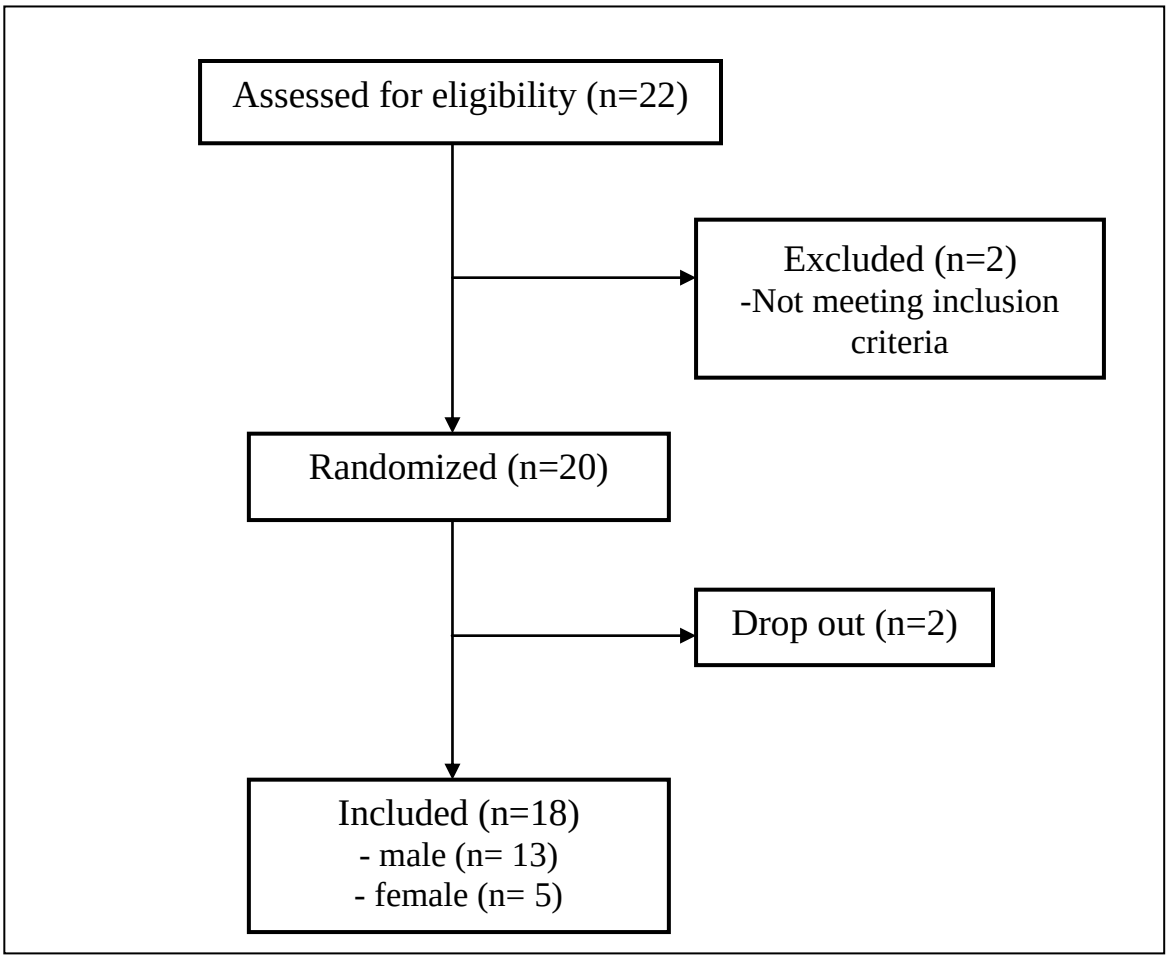

Figure 1. *Design of the study*

The participants' mean ± S.D. age (years), weight (lbs) and height (inch) were 23± 6 years, 164 ±28 Ibs, and 67 ± 3 inch.

Demographics and baseline characteristics of the overall population are presented in **table 3**.

Sex	**M**	**M**	**F**	**M**	**F**	**M**	**M**	**M**	**F**	**M**	**M**	**M**	**M**	**M**	**F**	**F**	**M**	**M**
Age	25	40	38	26	22	24	25	32	31	31	31	27	24	23	39	30	21	22
Heigh (inches)	69	67	61	65	64	69	70	65	64	70	69,5	63	72	68	65,5	64	75	69
Weight (lbs)	211	155	135	135	155	150	200	150	130	200	165	158	210	180	140	135	205	140
Ethnicity	AA	C	C	C	C	C	AA	C	C	C	C	A	C	C	C	AA	C	AA/PI

Table 3*. Patient's characteristics.*

There were no statistically significant differences in the baseline values of QT intervals, QTc, HR, SBP and DBP of participants.

Terms single dose is influence of 5-Hour ENERGY® drink after day 1, 7 days is influence after 7 days of chronically use and all days represent all datas obtained in study.

4.1. Effect of 5- hour Energy®drink on QTc interval

QTc intervals were corrected using Bazett's and Friderica's formula.

Average QT intervals measured in lead II and corrected with Bazett's formula for all days of study are shown in figure 2. The QTc of subjects who took 5-Hour ENERGY® drink is elevated on BL and 3 hours after consuming. For 1st hour after consummation and after 5 hours the QTc was reduced. But those results were not statistically significant differences. (**Fig. 2 and Table. 4**)

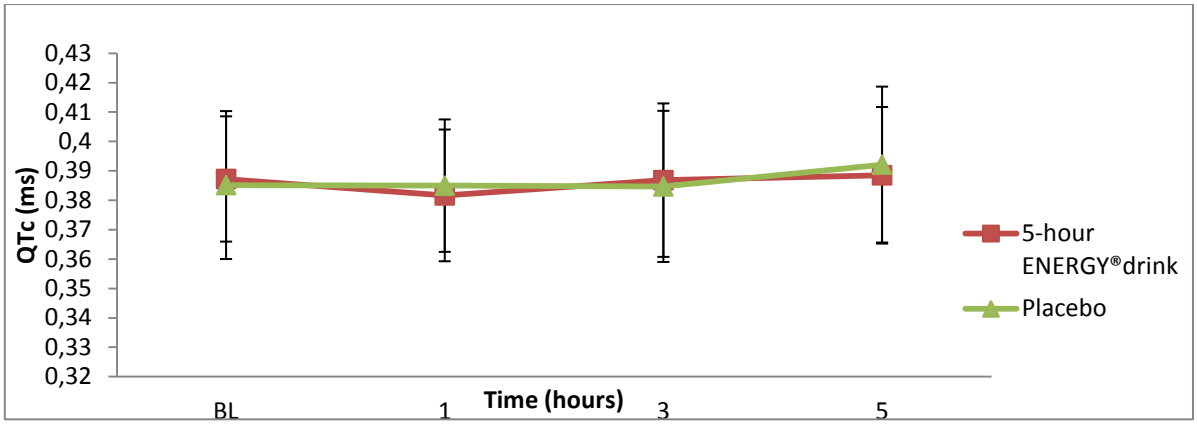

Figure 2. *Average QTc corrected with Bazett's formula for all days*

	Placebo (ms)	5-hour ENERGY®drink (ms)	p value
BL	385±25	387±21	0,551
1 hour	385±23	382±22	0,397
3 hour	385±26	387±26	0,583
5 hour	392±27	388±23	0,261

Table 4. *Average QTc corrected with Bazett's formula for all days*

When compared to placebo, 5-Hour ENERGY® drink does not significantly changing QTc after single dose on days 1 and 15. Results are shown in **Fig 3** and **Table 5** and there is small reducing of QTc after drinking 5-Hour ENERGY® drink but they not appear to clinical significance.

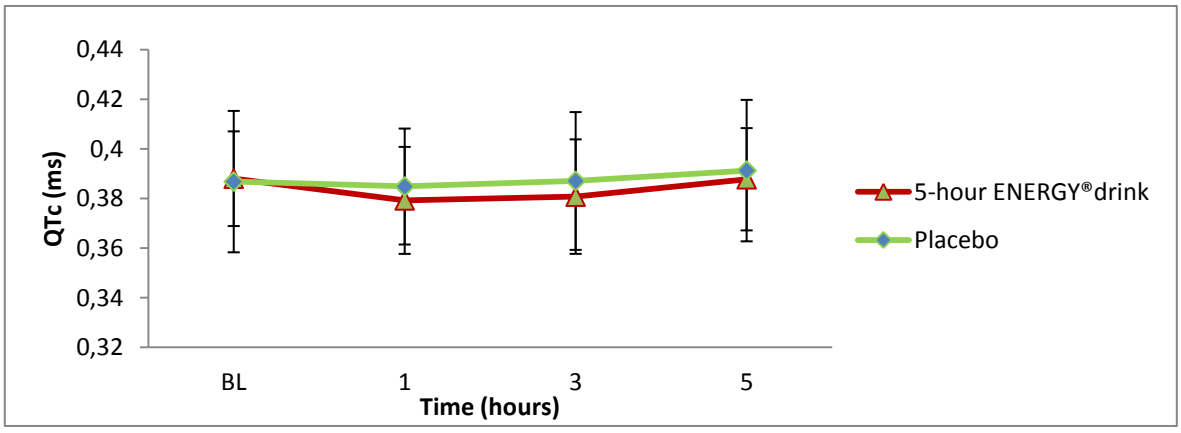

Figure 3. *Average QTc corrected with Bazett's formula after day 1*

	Placebo(ms)	5-hour ENERGY®drink(ms)	p value
BL	387±28	388±19	0,821
1 hour	385±23	379±22	0,368
3 hour	387±28	387±23	0,163
5 hour	391±28	388±21	0,436

Table 5. *Average QTc corrected with Bazett's formula after day 1*

Differences between 5-Hour ENERGY® drink and placebo after 7 days of consuming were not statistically significant (P> 0,05). There was small increase of QTc after 3rd hour of consuming. The details are presented in **Figure 4** and **Table 6**.

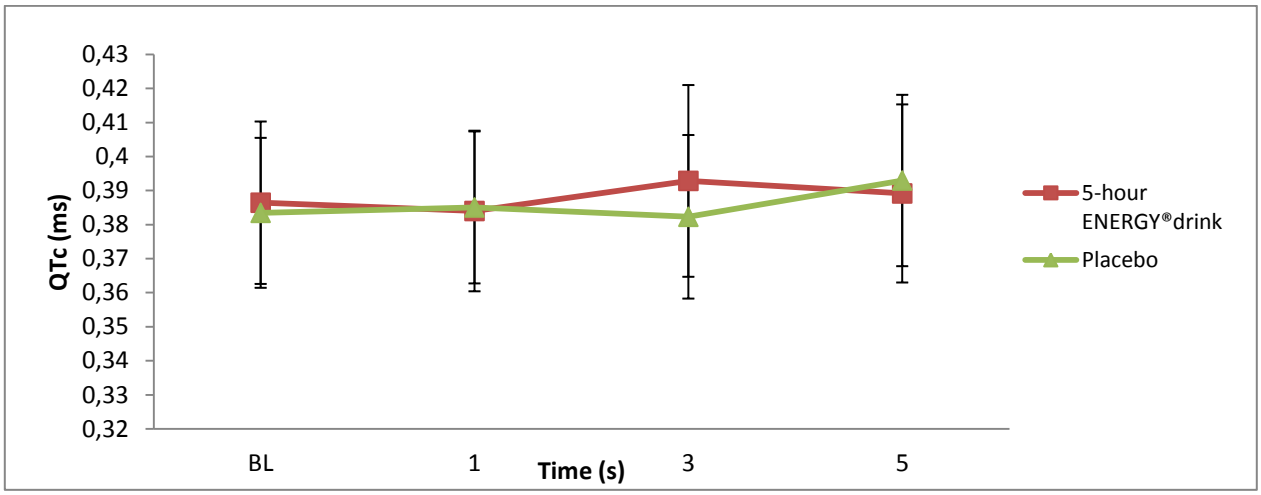

Figure 4. *Average QTc corrected with Bazett's formula after 7 days*

	Placebo(ms)	5-hour ENERGY®drink(ms)	p value
BL	383±22	386±24	0,542
1 hour	385±22	384±24	0,834
3 hour	382±24	393±28	0,086
5 hour	393±25	389±26	0,437

Table 6. *Average QTc corrected with Bazett's formula after 7 days*

Subsequently, the QT interval's value corrected with Friderica's formula was analyzed. We compared all days after consuming 5-Hour ENERGY® drink and placebo and there is small increase of QTc immediately after consuming and after 3 hours but overall no statistically significant differences (P>0,05) were found. The results are presented in **Fig 5** and **Table 7**.

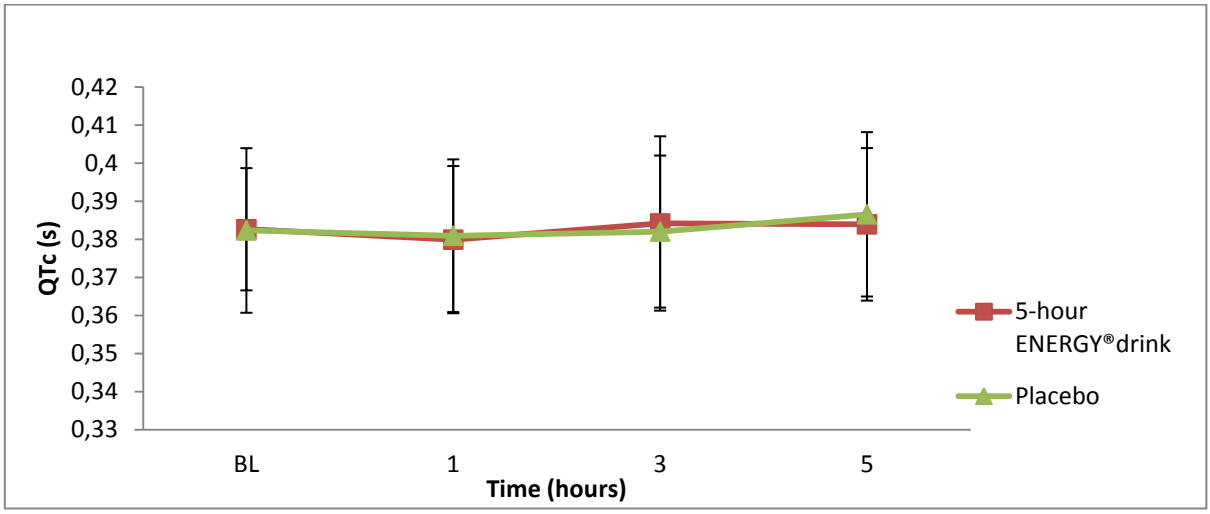

Figure 5. *Average QTc corrected with Friderica's formula for all days*

	Placebo(ms)	5-hour ENERGY®drink(ms)	p value
BL	382±22	383±16	0,904
1 hour	381±20	380±19	0,736
3 hour	382±20	384±23	0,431
5 hour	387±22	384±20	0,267

Table 7. *Average QTc corrected with Friderica's formula for all days*

There were no statistically significant differences (P>0,05) for compared QTc values after 1st and 15th day (**Figure 6**, **Table 8**). There is seen small reduction after 1st hour after consummation.

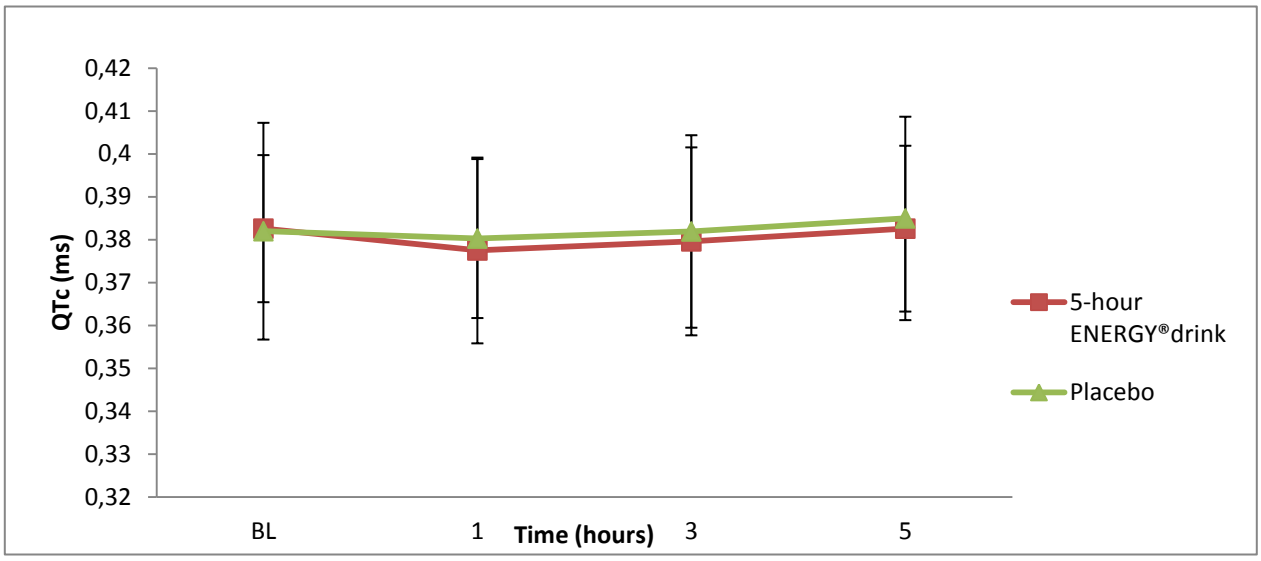

Figure 6. *Average QTc corrected with Friderica's formula after day 1*

	Placebo(ms)	5-hour ENERGY®drink(ms)	p value
BL	382±25	383±17	0,884
1 hour	380±19	377±22	0,564
3 hour	382±22	380±22	0,501
5 hour	385±24	383±19	0,451

Table 8. *Average QTc corrected with Friderica's formula after day 1*

In QT intervals corrected with Friderica's formula were no statistically significant differences observed between QTc values after drinking placebo and 5-Hour ENERGY® drink. However, higher value was seen in 3rd hour after drinking 5-Hour ENERGY® drink, but that difference wasn't significant (P>0,05). Results are shown in **Figure 7** and **Table 9**.

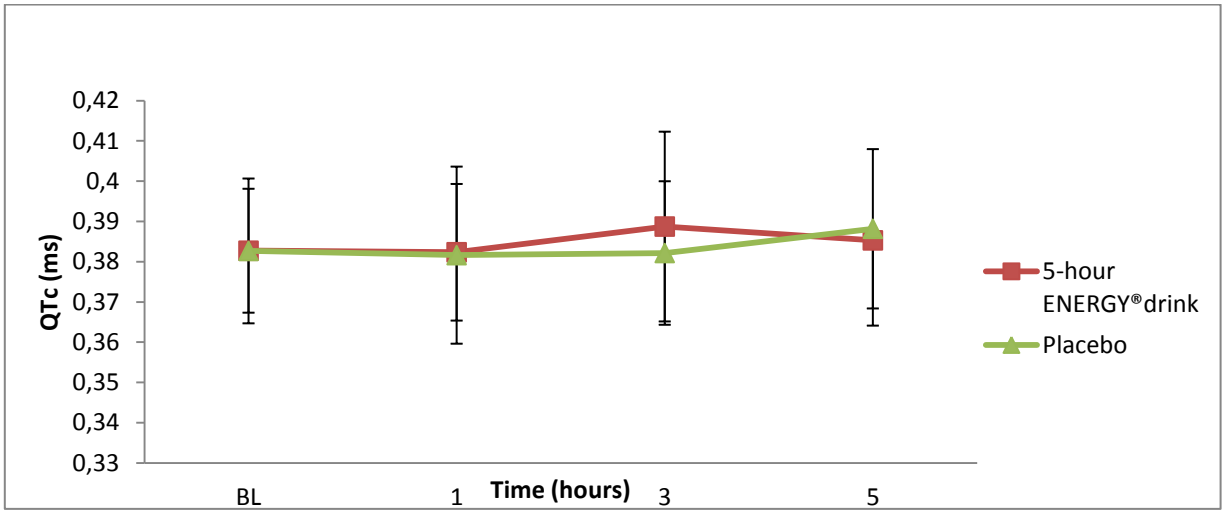

Figure 7. *Average QTc corrected with Friderica's formula after 7 days*

	Placebo(ms)	5-hour ENERGY®drink(ms)	p value
BL	383±18	383±15	0,989
1 hour	382±22	382±17	0,857
3 hour	382±18	389±24	0,119
5 hour	388±20	385±21	0,433

Table 9. *Average QTc corrected with Friderica's formula after 7 days*

4.2. Effect of 5- hour Energy®drink on QT interval

QT intervals values with P vales for all days are shown in **Table 10** and **Figure 8**.

No statistically significant differences were found immediately after drinking placebo or active (P=0,329), one hour after (P=0,266), three hours after (P=0,429) and 5 hours after (P=0,839).

The average QT values after drinking 5-Hour ENERGY® drink were for BL 374±19ms, for 1st hour 377±23 ms, for 3 hours after 379±26 ms and after 5 hours was 376±25 ms. The average QT values after drinking placebo were for BL= 382±24 ms, 1h =379±23 ms, 3h= 393±19 ms, 5 h=389±19 ms.

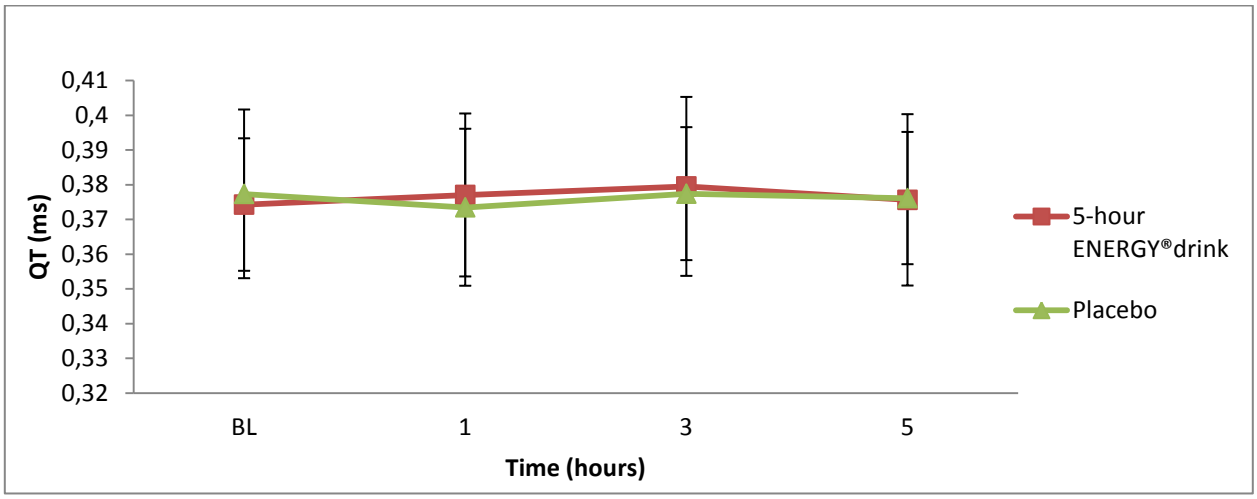

Figure 8. *Average QT after 5-Hour ENERGY® drink and placebo for all days*

	Placebo(ms)	5-hour ENERGY®drink(ms)	p value
BL	382±24	374±19	0,330
1 hour	379±23	377±23	0,267
3 hour	393±19	379±26	0,493
5 hour	389±19	376±25	0,839

Table 10. *Average QT after 5-Hour ENERGY® drink and placebo for all days*

In **Figure 9** and **Table 11** are shown result of QT interval beetwen drinking 5-Hour ENERGY® drink and placebo after 1st. No statistically significant differences were after drinking placebo or active, one hour after (P=0,514), three hours after (P=0,215) and 5 hours after (P=0,940).

The average QT values after drinking 5-Hour ENERGY® drink for BL was 372±21ms, for 1st hour 374±26 ms, for 3 hours after 378±26 ms and after 5 hours was 373±26 ms. The average QT values after drinking placebo were for BL= 373±28 ms, 1h =372±21 ms, 3h= 372±23 ms, 5 h=373±22 ms.

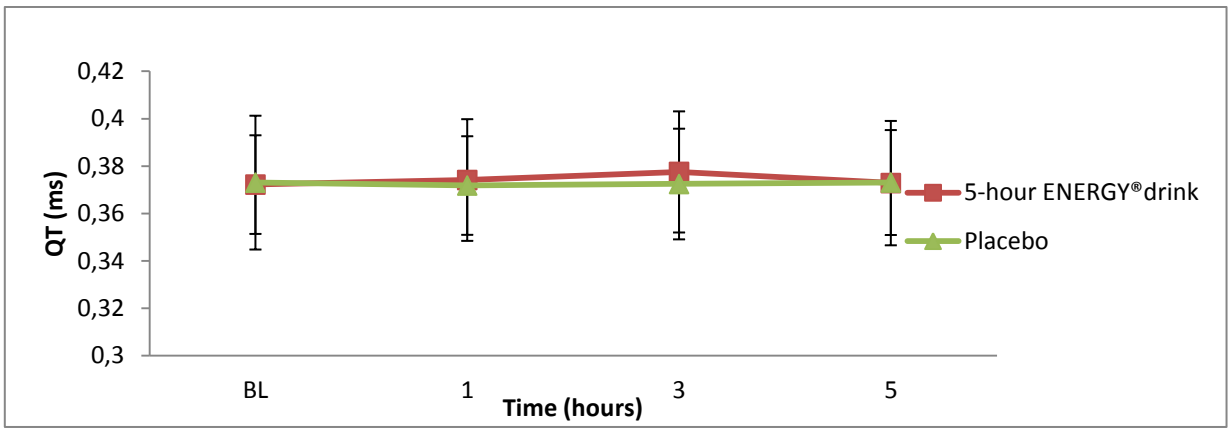

Figure 9. *Average QT after 5-Hour ENERGY® drink and placebo on day 1*

	Placebo(ms)	5-hour ENERGY®drink(ms)	p value
BL	373±28	372±21	0,860
1 hour	372±21	374±26	0,514
3 hour	372±23	378±26	0,215
5 hour	373±22	373±26	0,941

Table 11. *Average QT after 5-Hour ENERGY® drink and placebo on day 1*

The measured QT intervals after 7th days are presented in **Figure 10** and **Table 12**. We compared the QT interval after consuming 5-Hour ENERGY® drink and placebo and we determined that there were no statistically significant differences between them (BL P=0,0221; 1h P= 0,382; 3h= 0,835; 5 h P= 0,844).

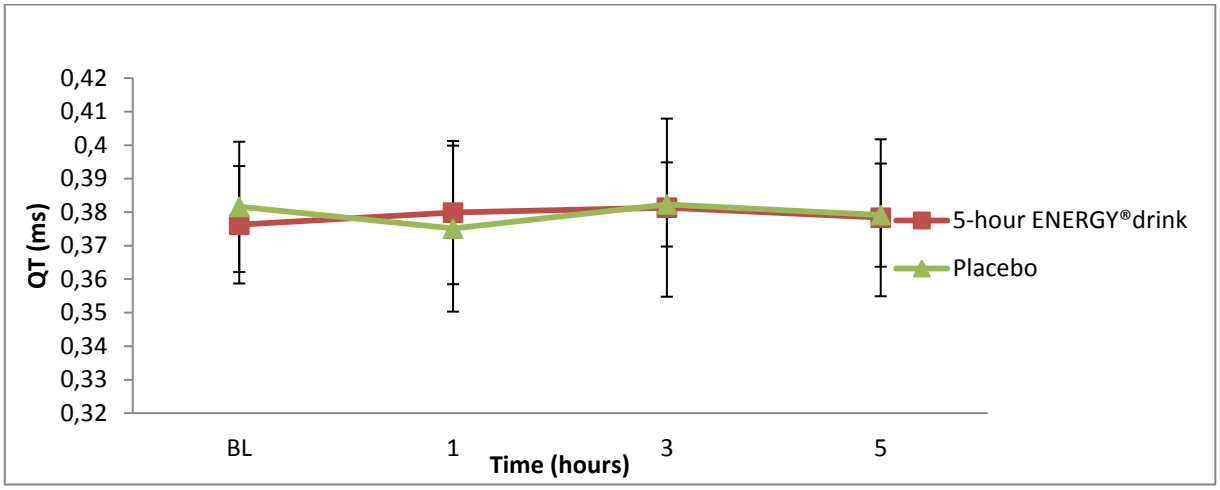

Figure 10. *Average QT after 5-Hour ENERGY® drink and placebo on day 7*

The average QT values after drinking 5-Hour ENERGY® drink for BL was 376±18, for 1st hour 380±21 ms, for 3 hours after 381±17 ms and after 5 hours was 378±23 ms. The average QT values after drinking placebo were for BL= 382±19 ms, 1h =375±25 ms, 3h= 382±13 ms, 5 h=379±15 ms.

	Placebo(ms)	**5-hour ENERGY®drink(ms)**	**p value**
BL	382±19	376±18	0,221
1 hour	375±25	380±21	0,382
3 hour	382±13	381±17	0,836
5 hour	379±15	378±23	0,845

Table 12. *Average QT after 5-Hour ENERGY® drink and placebo on day 7*

4.3. Effect of 5- hour Energy®drink on heart rate

Effect of 5- hour Energy®drink on heart rate

The effects of 5- hour Energy®drink or placebo on heart rate during the our study are presented in **Figure 11**. One hour after consumation 5-Hour ENERGY®drink, heart rate was 62±8 bpm and 64±8 bpm after placebo.Three hours after HR was 63±8 bpm for 5- ED and 62±9 bpm for placebo and five hours later, 64±9 bpm for 5-Hour ENERGY® drink and 65±7 bpm for placebo. However, no statistically significant differences were found after drinking 5-Hour ENERGY® drink during all study. The effects of 5-Hour ENERGY® drink on HR differed only minimally. All datas with p- values are presented in **Table 13.**

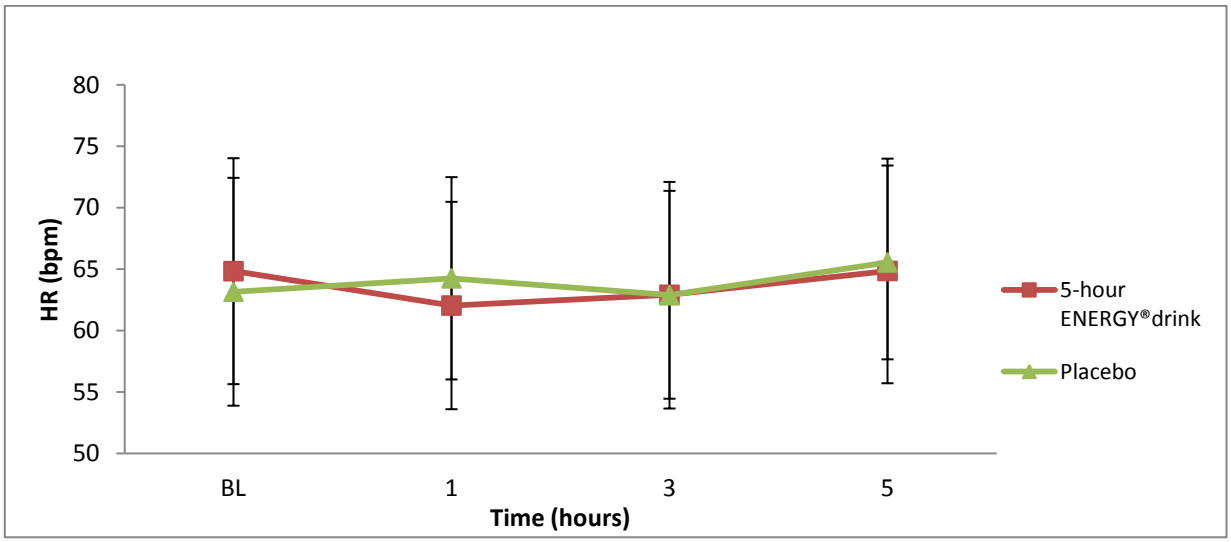

Figure 11. *Average HR after 5-hour Energy®drink for all days*

	5-hour ENERGY®drink(bpm)	Placebo(bpm)	p- value
BL	64±9	63±9	0,276
1 hour	62±8	64±8	0,144
3 hour	63±8	62±9	0,983
5 hour	64±9	65±7	0,598

Table 13. *Average HR after 5-Hour Energy®drink and placebo for all days*

In **Figure 12**. are shown results of effects 5- hour Energy® drink or placebo on heart rate on day 1. After 1 hour HR was 62±5 bpm after consuming 5-Hour ENERGY® drink and 65±10 bpm after placebo. Three hours later HR decreased markedly after administration of 5-Hour ENERGY® drink (p= 0,04). HR was 61±7 bpm for 5-Hour ENERGY® drink and 65±10 bpm for placebo. But after 5 hours, there were no statistically significant differences (HR: 65±9 bpm for 5-Hour ENERGY® drink and 66±8 bpm for placebo (**Table 14**)).

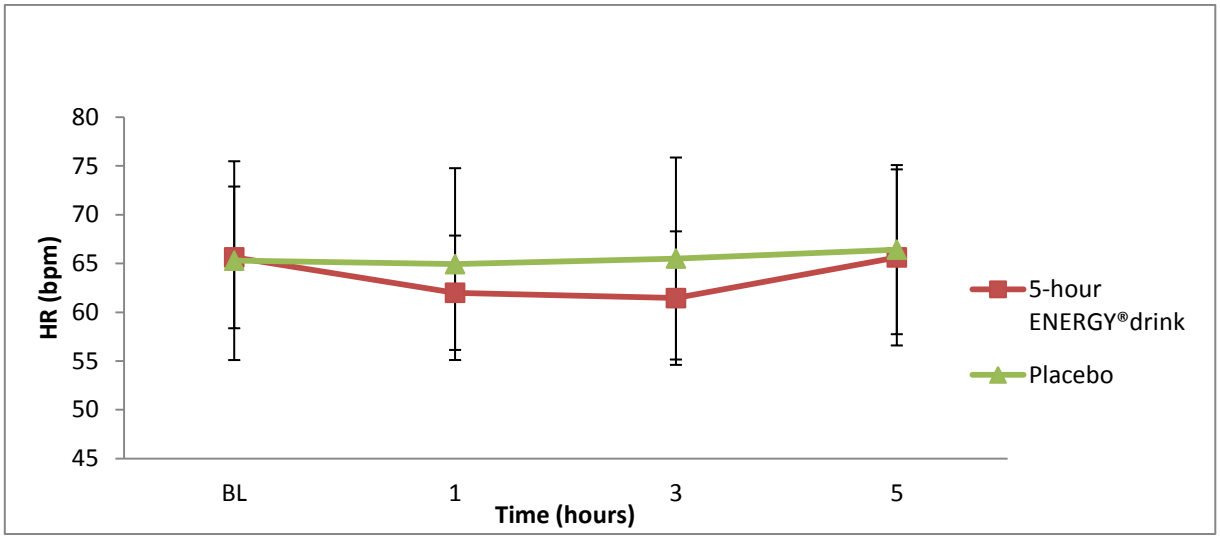

Figure 12. *Average HR after 5-hour Energy®drink and placebo after day 1*

	5-hour ENERGY®drink(bpm)	Placebo(bpm)	p- value
BL	65±7	65±10	0,881
1 hour	62±5	65±10	0,111
3 hour	61±7	65±10	0,041
5 hour	65±9	66±8	0,670

Table 14. *Average HR after 5-Hour Energy®drink and placebo on day 1*

The effects of 5-Hour ENERGY® drink or placebo on heart rate after 7 days are shown in **Figure 13**. One hour after consumation 5-Hour ENERGY® drink, heart rate was 62±10 bpm and 63±6 bpm after placebo.Three hours after consumation HR was 64±10 bpm for ED and 60±7 bpm for placebo and five hours later, 64±9 bpm for 5-Hour ENERGY® drink and 64±7 bpm for placebo. However, no statistically significant differences were found after drinking 5-Hour ENERGY® drink during all study. The effects of 5-Hour ENERGY® drink on HR differed only minimally. All datas are presented in **Table 15.**

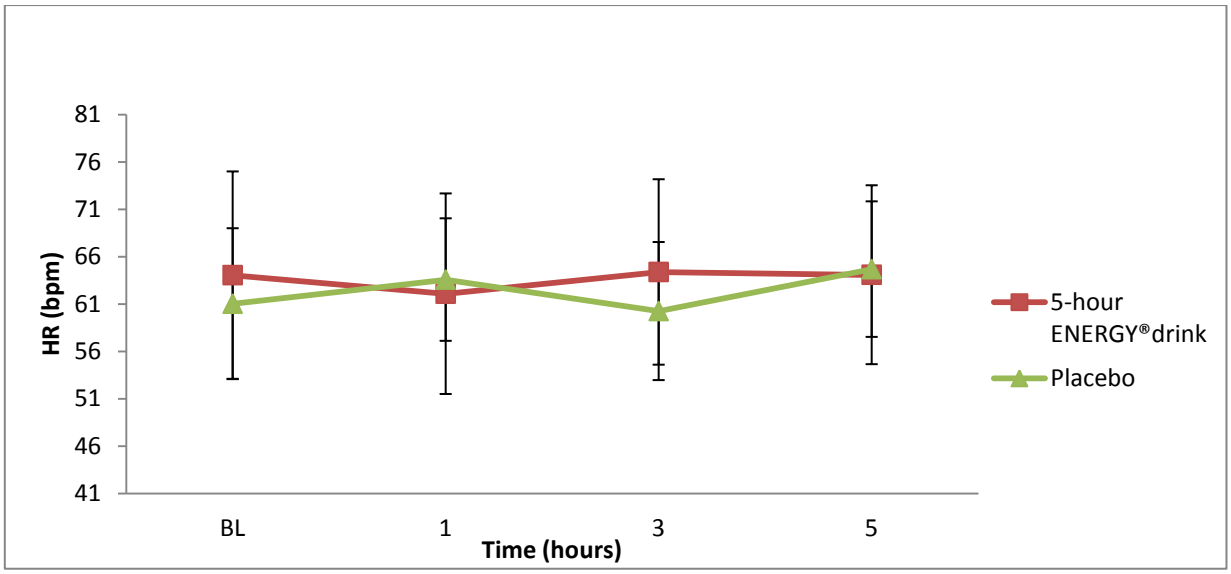

***Figure 13**. Average HR after 5-hour Energy®drink and placebo after 7 days*

	5-hour ENERGY®drink(bpm)	Placebo(bpm)	p- value
BL	64±11	61±8	0,168
1 hour	62±10	63±6	0,548
3 hour	64±10	60±7	0,114
5 hour	64±9	64±7	0,759

***Table 15.** Average HR after 5-Hour Energy®drink and placebo after 7 days*

4.4. Effect of 5- hour Energy®drink on blood pressure

The largest blood pressure response to 5-Hour ENERGY® drink we expected during chronically consuming 5-Hour ENERGY® drink for all days. SBP response for all days, 1 hour after intake (p=0,095, 119±7 mmHg for placebo, 123±9 mmHg for 5-Hour ENERGY® drink) was not statistically significant difference, but 3 and 5 hours after intake there were statistically significant differences (after 3 h: p=0,031, 118±8 mmHg (placebo) and 124±10 mmHg (5-Hour ENERGY® drink); after 5 h: p= 0,015, 116±8 mmHg (placebo) and 122±9 mmHg (5-Hour ENERGY® drink)). Average maximal SBP for all patients after receiving placebo were 121±7 and 126±10 mmHg after drinking 5-Hour ENERGY® drink with p= 0,026. Those changes were statistically significant. SBP maximal difference for all days after placebo was 3±4 mmHg and 6±5 mmHg after 5-Hour ENERGY® drink (p=0,003). All results are presented in **Figure 14** in **Table 16**.

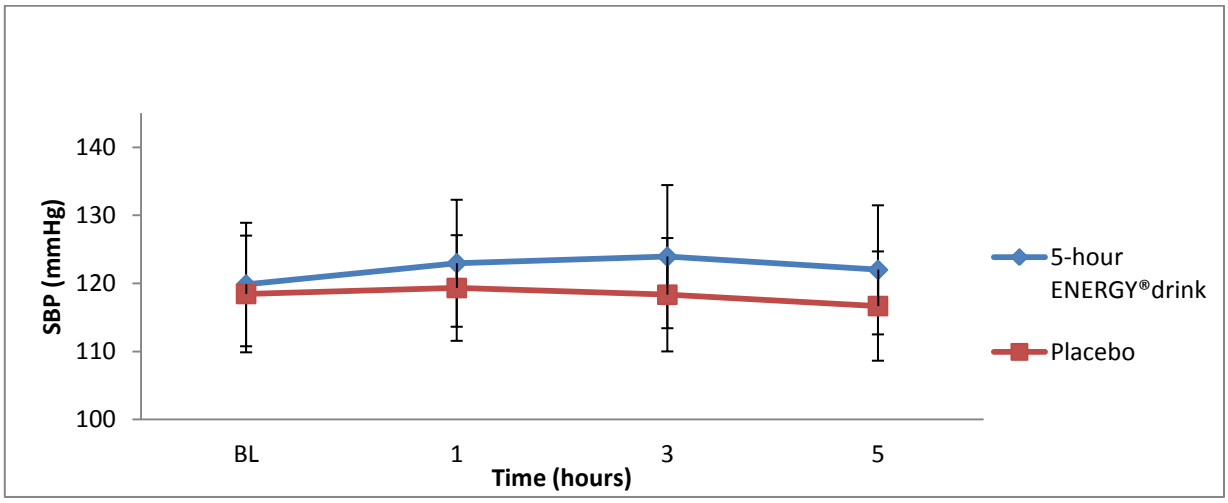

Figure 14. *Average SBP after 5-hour Energy®drink and placebo for all days*

	5-hour ENERGY®drink(mmHg)	Placebo(mmHg)	p- value
BL	119±9	118±8	0,512
1 hour	123±9	119±7	0,095
3 hour	124±10	118±8	0,031
5 hour	122±9	116±8	0,015
Max SBP	126±10	121±7	0,026
Max diff	6±5	3±4	0,003

Table 16. *Average SBP after 5-Hour Energy®drink and placebo for all days*

When we were assessed the impact of 5-Hour ENERGY® drink on SBP on day 1, we did not found statistical significant difference in 1 and 3 hours after drinking 5-Hour ENERGY® drink (after 1 h: p= 0,335, mean(±)SD for placebo 118±9 mmHg, 121±9 mmHg for 5-Hour ENERGY® drink; after 3 h: p= 0,118, 118±9 mmHg for placebo and 124± 10 mmHg for 5-Hour ENERGY® drink). With p= 0, 065, 116±9 mmHg for placebo and 122±9 mmHg for 5-Hour ENERGY® drink, differences of SBP 5 hour after intake were not statistically significant. Average maximal SBP was 126±11 mmHg after 5-Hour ENERGY® drink and 121±8 mmHg for placebo with p=0,149 that is not significant.

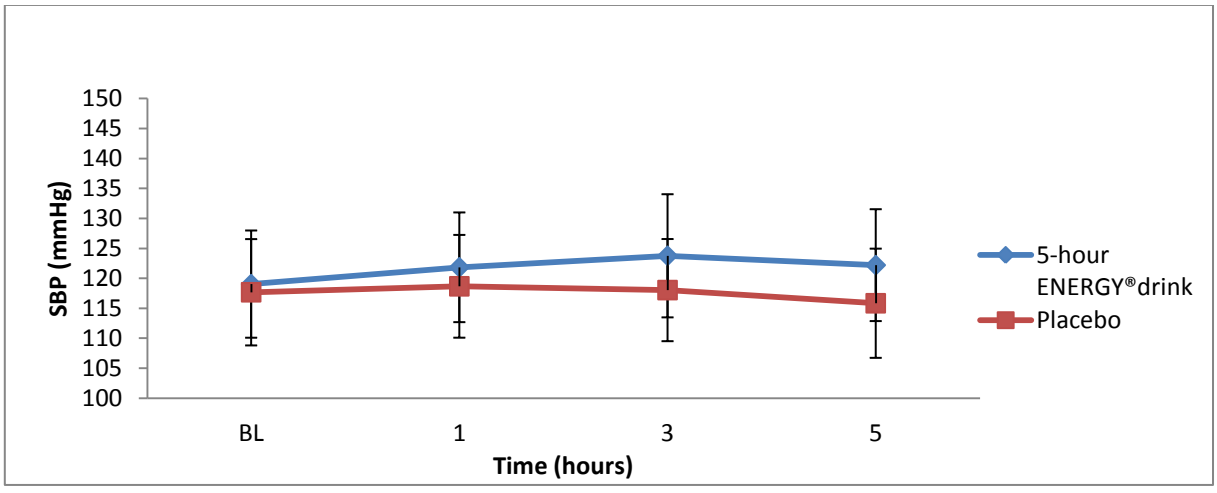

Figure 15. *Average SBP after 5-hour Energy®drink and placebo after day 1*

	5-hour ENERGY®drink(mmHg)	Placebo(mmHg)	p- value
BL	119±9	118±9	0,686
1 hour	122±9	119±9	0,335
3 hour	124±10	118±9	0,118
5 hour	122±9	116±9	0,065
Max SBP	126±11	121±8	0,149
Max diff	7±6	4±4	0,062

Table 17. Average SBP after 5-Hour Energy®drink and placebo on day 1

After seven days continuous drinking 5-Hour ENERGY® drink twice per day we found there were no significant difference (at 1 hour: p=0,181, mean±SD for placebo 120±7 mmHg and for 5-Hour ENERGY® drink 124±9). The values at 3 hours after consuming placebo or 5-Hour ENERGY® drink were p=0,155 119±9 mmHg for placebo, 124±11mmHg for active. However, there were no significant difference and the same was for data's at 5 hours after consuming placebo or 5-Hour ENERGY® drink (p=0,137, 118±7 mmHg, 122±10 mmHg for active). Maximal difference between SBP after 7 days was 3±5 mmHg for placebo and 6±5 mmHg for active with p=0,023 that is statistical significant difference. The results are shown in **Figure 16** and **Table 18**.

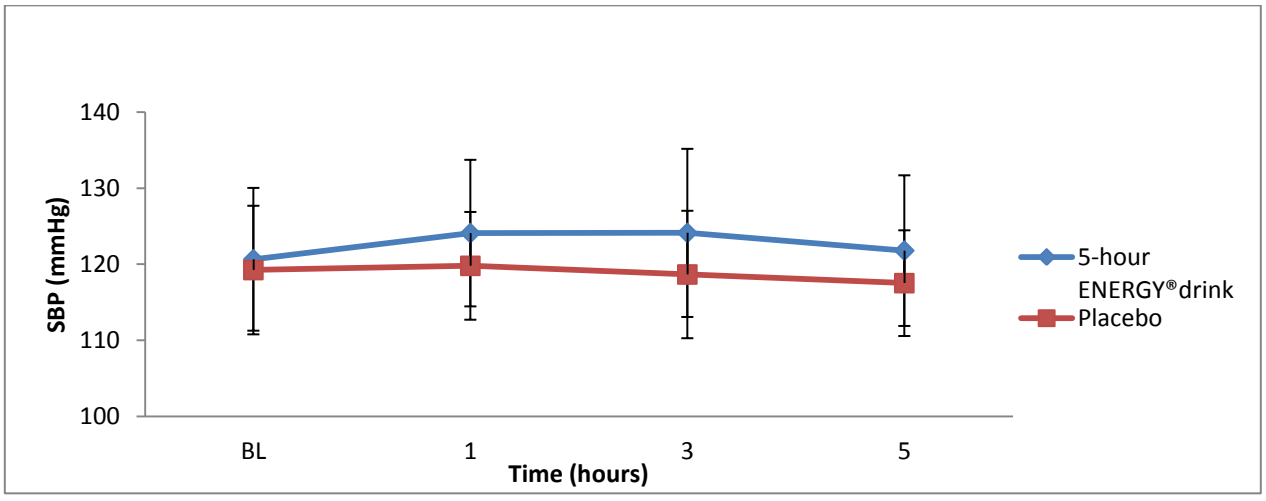

Figure 16. *Average SBP after 5-hour Energy®drink and placebo after 7 days*

	5-hour ENERGY®drink(mmHg)	Placebo(mmHg)	p- value
BL	121±9	119±9	0,601
1 hour	124±10	120±7	0,181
3 hour	124±11	119±9	0,155
5 hour	122±10	118±7	0,137
Max SBP	127±10	122±6	0,098
Max diff	6±5	3±5	0,023

Table 18. *Average SBP after 5-Hour ENERGY®drink and placebo after 7 days*

Consuming 5-Hour ENERGY® drink versus placebo affected on DBP data's and changes were statistically significant at 1 and 5 hours after consuming for time rang all days (1 h: p= 0,017, 77±6 mmHg for placebo, 82±8 mmHg for active, 5 h: p= 0,024, 75±6 mmHg for placebo and 79±7 mmHg for 5-Hour ENERGY® drink). In 3 hours we did not found statistically significant differences (p= 0,065, 77±8 mmHg for placebo, 80±7 mmHg for 5-Hour ENERGY® drink). Comparison two groups are presented in **Figure 17** and **Table 19**. The average maximal DBP between placebo group was 78±7 mmHg and between active group 83±7 mmHg with p=0,017 that is statistical significant difference. Maximal differences among DBP in placebo vs. active group were 2±4 mmHg, 5±5 mmHg with p=0,003.

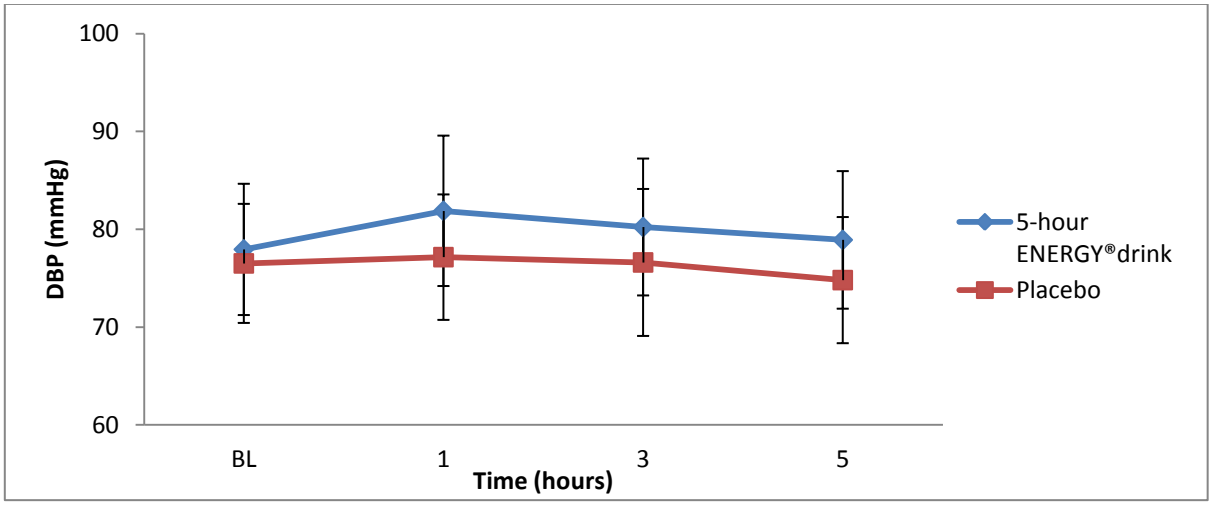

Figure 17. *Average DBP after 5-hour Energy®drink and placebo for all days*

	5-hour ENERGY®drink(mmHg)	Placebo(mmHg)	p- value
BL	78±7	77±6	0,378
1 hour	82±8	77±6	0,017
3 hour	80±7	77±8	0,065
5 hour	79±7	75±6	0,024
Max DBP	83±7	78±7	0,017
Max diff	5±5	2±4	0,003

***Table 19**. Average DBP after 5-Hour Energy®drink and placebo for all days*

Change of DBP 1 hour after drinking 5-Hour ENERGY® drink on day 1 (**Figure 18 and Table 20**) were not significantly with p= 0,068, 77±6 mmHg for placebo and 82±8 mmHg for active. As well, changes after 3 and 5 hours were not significantly (3 h: p= 0,245, 77±8 mmHg for placebo, 80±8 mmHg for active; 5 h: p= 0,071, 75±8 mmHg for placebo and 80±7 mmHg). Maximal average DBP was 78±7 mmHg after placebo and 83±8 mmHg with p= 0,111. Maximal differences of DBP in placebo vs. active group were 1±4 mmHg and 5±4 mmHg with p=0,001 that is significantly change.

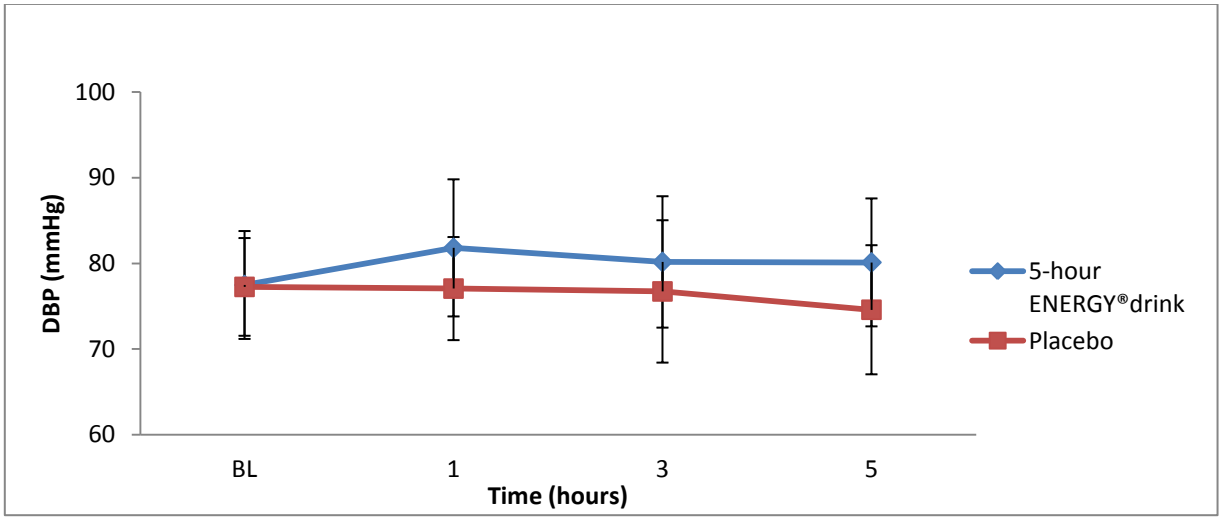

***Figure 18.** Average DBP after 5-hour Energy®drink and placebo after day 1*

	5-hour ENERGY®drink (mmHg)	Placebo (mmHg)	p- value
BL	77±6	77±6	0,922
1 hour	82±8	77±6	0,068
3 hour	80±8	77±8	0,245
5 hour	80±7	75±8	0,071
Max DBP	83±8	78±7	0,111
Max diff	5±4	1±4	0,001

Table 20. *Average DBP after 5-Hour Energy®drink and placebo after 1 day*

Assessing impact of 5-Hour ENERGY® drink on DBP after 7 days, we did not found statistical significant difference in 1, 3 and 5 hours after drinking 5-Hour ENERGY® drink (after 1 h: p= 0,129, mean(±)SD for placebo 77±7 mmHg and for 5-Hour ENERGY® drink 82±8 mmHg; after 3 h: p= 0,159, mean(±) for placebo 76±7 mmHg and for 5-Hour ENERGY® drink 80± 6 mmHg, after 5 h: p= 0,198, mean(±) for placebo 75±5 mmHg and for 5-Hour ENERGY® drink 78± 7 mmHg). Results are presented in **Figure 19**. Maximal average DBP was 78±6 mmHg after placebo and 83±7 mmHg with p= 0,089. Maximal differences of DBP in placebo vs. active group were 2±3 mmHg and 4±5 mmHg with p=0,216 that were not statistically significance changes.

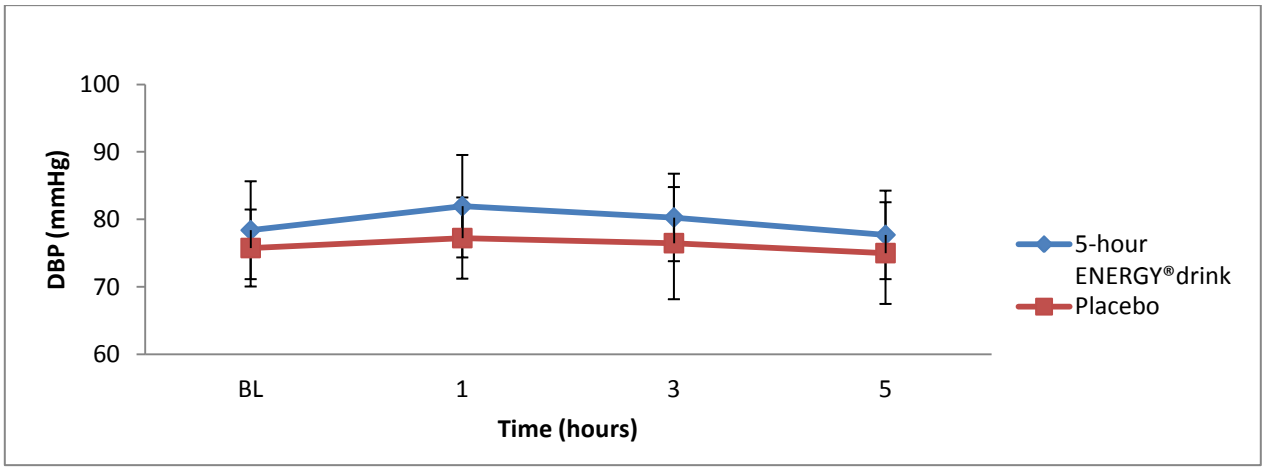

Figure 19. *Average DBP after 5-hour Energy®drink and placebo after 7 days*

	5-hour ENERGY®drink(mmHg)	Placebo(mmHg)	p- value
BL	78±7	76±7	0,273
1 hour	82±8	77±7	0,129
3 hour	80±6	76±7	0,159
5 hour	78±7	75±5	0,198
Max DBP	83±7	78±6	0,089
Max diff	4±5	2±3	0,216

Table 21. *Average DBP after 5-Hour Energy®drink and placebo after 7 days*

4.5. Automatic vs. Manual

For the depicting the relationship between manual and automatic measured average QTc and average HR we used the pearson correlation coefficient

Correlation coefficient for QTc was 0,754 and for HR 0,882.

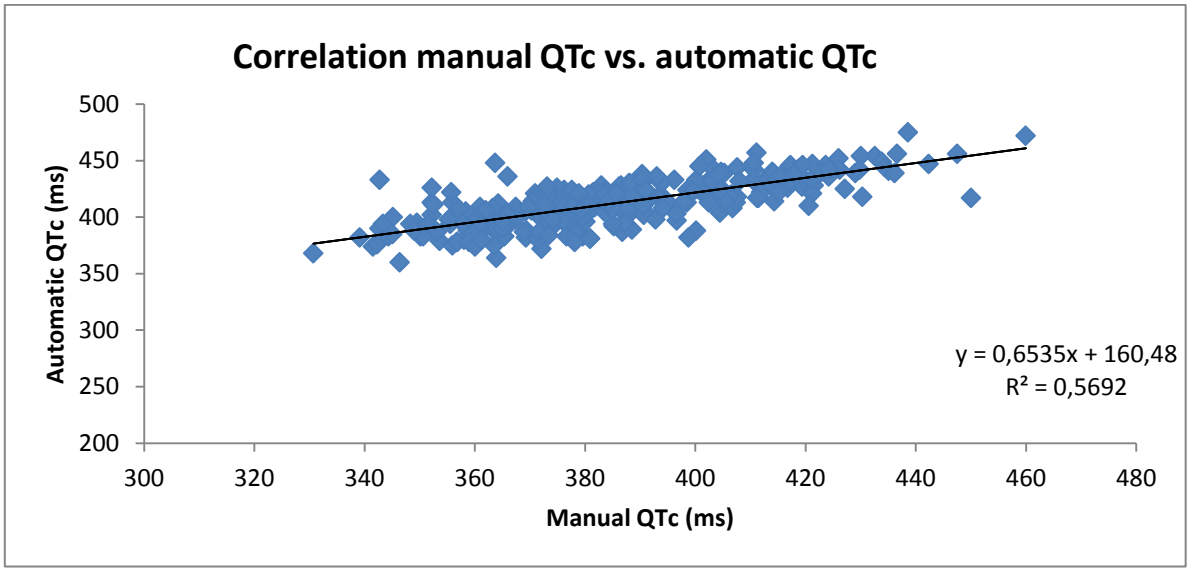

Figure 20. *Scatter plot showing the correlation between QTc measured automatic and manual*

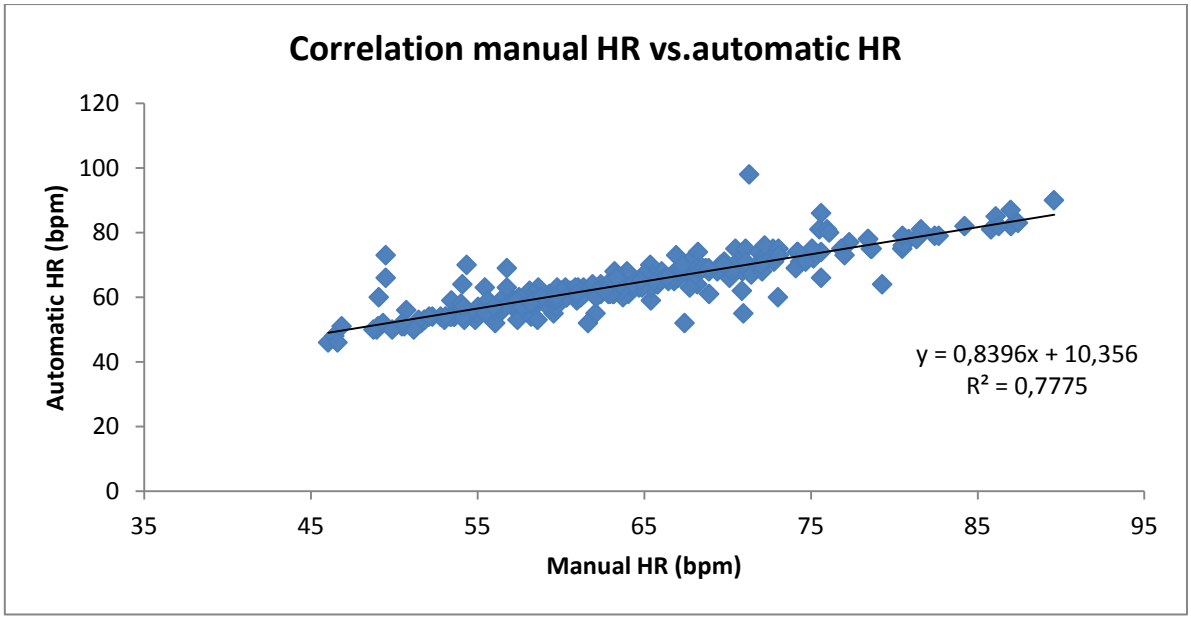

Figure 21. *Scatter plot showing the correlation between HR measured automatic and manual*

4.6. Adverse effects

All participants recived two bottles of 5-Hour ENERGY® drink a day spaced out. That is maximum recomended dose. 17 subjects (94,4%) after drinking 5-Hour ENERGY® drink reported a treatment- adverse drug effects.

The most frequently reported adverse events within the consummation 5-Hour ENERGY® drink calculated in relation to the total number of adverse effects were: cardic effects (20%) such as chest pressure, heart racing and heart rate increasing; somnolence (20%); GI upset (16%); headache (12%); facial flushing (8%); jittery (8%); dizzy (4%), sleeplessness (4%), nausea (4%), diarrhea (4%).

The most frequently reported adverse events after consummation placebo were: cardic effects (36%); headache, GI upset; sleeplessness (18%).

Also there were few reported adverse events within consummation placebo. The results are shown in **Table 21.**

	5-hour ENERGY®drink	**Placebo**
Cardic	5	4
Headache	3	2
GI upset	4	2
Sleepiness	5	1
Facial flushing	2	0
Dizzy/ HA	1	0
Jittery	2	0
Sleeplessness	1	2
Nausea	1	0
Diarrhea	1	0
Total	25	11

Table 21.*Reported adverse events*

5. DISCUSSION

Many people consume EDs but the adverse effects of the latter are still not well known and controversial. Since these drinks are readily available and qualitative composition is not controlled by the Food and Drug Administration (FDA), assessing their effects is important.

5-Hour ENERGY® drink

The present study has proven acute effects of 5-Hour ENERGY® drink on HR and blood pressure. In our study, we did not found significant differences between QTc intervals of healthy subjects after ingestion of a single dose of 5-Hour ENERGY® drink or placebo. Because the QT is dependent on the heart rate, which means the faster the HR, the shorter the QT interval, we corrected all measured values by using Bazett's and Friederica formulas.

Findings that there were no significant differences on QTc mean good news for the regular users of 5-Hour ENERGY® drink, under the condition that they are healthy, like our volunteers.

This statement does not apply for cohort groups that are older than 40, for those hypertensive patients older or younger than 40 with treated or not treated hypertension.

Caffeine consumers might become tolerant to caffeine's effects or maybe become even more sensitive if their daily caffeine consummation is high. As caffeine tolerance may develop within just a few days, it could also appear within a few hours (8, 54). In one study, they found that a chronic daily administration of caffeine in average amounts of 60-70mg/kg per day resulted in caffeine tolerance (55, 56). Another study showed that the development of tolerance is different from person to person (57).

The following parameter that we researched was the HR. Higher HR at the resting state is a risk for heart disease. Relating to the other studies, we expected a slight decrease in HR (74). At 3 hours after drinking a single dose of 5-Hour ENERGY® drink, there was a statistically significant decrease in HR. It was probably because of the direct vagal stimulation or due to an effect on the sinoatrial node (58, 59).

Another possible explanation of a HR decrease might be the baroreflex that is reflexively lowering HR while trying to establish a normal BP. Although the HR is significantly decreased 3 hours after single dose, it didn't decrease significantly after 7 days of chronically drinking 5-Hour ENERGY® drink in time intervals 1, 3 and 5 hours after consumption. It is very interesting why our volunteers reported feeling of fast heart beat as an adverse effect but we did not found any significant differences. Even if the heart rate went down in 3 hours, the blood pressure went up because of compensatory mechanism.

According to the effect of the 5-Hour ENERGY® drink to BP, the increase is not large, but risk for some cardiovascular disease is still possible. In one study, they said that also a small increase in BP, like 2mm Hg, can have an effect on cardiovascular disease outcomes (57).

In one study they found that two cups of coffee increase the BP for 5-10mm Hg (7).

Elevation of SBP is a more important indicator of cardiovascular events than DBP for middle-aged and older populations. But in young people (late adolescents), the DBP is a stronger indicator of cardiovascular events than SBP (62). Normal DBP is 80 mm Hg or less and values higher than 90 mm Hg are considered to be hypertension. Hypertension in adolescents is related with higher BP in later life.

In our study, the maximal DBP after consuming 5-Hour ENERGY® drink was 83 mm Hg. Relating to results from all of the days, it was statistically significant at 1 and 5 hours after consumption. The maximum elevation in SBP was also statistically significant, together with increase of SBP in 3 and 5 hours. The maximum SBP (126 mm Hg) that we found might be a sign of prehypertension state. SBP on day 1 and day 7 was not statistically significant and it was the same for DBP.

Possible reason for that pressure effect might be peripheral vasoconstriction.

Office blood pressure monitoring is an important standard for BP measurement, but the problem is that it is not good for long-term, chronic variability in BP (over 24 hours) (61, 62).

Our participants were healthy and young, without any cardiovascular diseases or history, and it is interesting that the DBP and the SBP change significantly. People who are not familiar

with family history of cardiovascular disease or those that still don't have any symptoms of high BP and other cardiovascular diseases should therefore be careful. Those who have any cardiovascular diseases in family history or high BP or any other cardiovascular diseases should be careful even more.

Hand measurement

We minimized possible errors during the clinical study. Because the quality of the ECG databases depends on the ECG machine, we used modern equipment with the capacity for digital signal processing; it was also serviced and calibrated. The reader of the ECGs was blinded during the study and all measured values were checked by another reader under blinded conditions.

Historically, hand measurement of ECGs was considered the gold-standard for analysis. With newer technology, using automated ECG versus manual ECG readings is controversial. While we used the 12- lead electrocardiograph (PageWriter Trim III, Philips Health-care, Andover, MA) as the standard in our study we also wanted to see the correlation between the two.
We used scatter plot for interpreting trends in data. Scatter plot with QTc show an uphill and positive correlation between automatic and manual measured QTc intervals. With Pearson's coefficient 0,754 we see there is a good relationship between our variables.
Dispersion of HR data points was smaller. The correlation coefficient for automatic vs. manually measured HR was 0,882 that is strong correlation between variables.

We can assume that there is no difference among measuring ECG automatically or manually. Our volunteers were reporting about ED related adverse effects. According to reports, 20% of them were connected with cardiovascular problems such as heart racing, feeling of increased HR and palpitation.

Adverse effects

Another very common adverse effect was sleepiness (20%). That is surprising because the 5-Hour ENERGY® drink is advertised like drink that help you get rid of sleepiness. A possible

cause for this adverse effect could be a big amount of sugar that causes a rapid decrease in blood sugar.
Gastrointestinal upset was also frequently reported by our volunteers. Facial flushing (also called niacin flush and skin redness symptoms, produced by niacin-caused vasodilatation) were reported in just 8%.
Subject that were receiving placebo reported about some adverse effects. This is commonly known as placebo effect.

Regulations of energy drinks

Today, any product that is containing caffeine can be called an ED. Caffeine is a drug 80% of people commonly consume every day. (63) Concern over the amounts of caffeine consumed in the form of ED is getting bigger by the day. We live a fast life and while people sleep less, their bodies still need real rest and energy. Because of that, a lot of people decide to take the magic energy sleep in a can.
Questions are: What exactly is an ED? What ingredients belong to ED cans? What is the real effect ED has on human body and human heart?
While there have been studies conducted that show the safety of some of the individual ingredients contained in 5-Hour ENERGY® drink, to our knowledge, there has been no clinical studies conducted to evaluate the cardiac effects of a 5-Hour ENERGY® drink as a unit.

Many of us are probably confused about product usage, ingredients and safety. The ED industry is definitely missing an important rule about the definition of an ED.
Being a part of modern way of living also brings, along with all of the benefits and comforts, confusion. Every day, we are bombed from different media (such as TV, radios, smartphones, and PCs) with lots of information about new products, new solutions, new diets, and new dietary supplements. It is hard to choose what to believe in. The ED industry is a part of this. Unfortunately, it is sometimes hard to be well informed about everything.
In grocery stores worldwide, the EDs are placed right next to other beverages like coca cola, juices, and mineral water. Their containers are colorful, with different photos and attractive messages designed to attract your attention. They have some slogans, like 5-Hour Energy® –

“no crash later”, “gives you wings” or “unleash the beast” and, in some cases, their names are associated with alcohol beverages (as Monster Cuba Lima) or with sexuality.
Just because of that, the perception that most people have is that the ED are, in fact, soft drinks and that they are healthy.

There is also a big confusion between the EDs and sports drinks. This could be a result of the fact that many ED companies act as main sponsors of different local sports events (motor races, Formula 1). Electrolytes and carbohydrates, the main ingredients of sports drinks, are not the same as caffeine and other stimulant drugs in ED (64). The source of this mess is strong marketing with very good advertisements sending the message that ED give you energy, and improve performance alertness (63). One big part of advertising ED is associated with nutrition, health, and physical activity, without giving right information about safety. It is also not rare that big ED companies use the terms “organic” and “all natural”.
In 2012, the 5-Hour ENERGY® drink company spent 194.6 million dollars for advertising their products (66).

The regulation of new drug entities is strongly regulated by FDA, and it is opposite to the regulation of the nutraceuticals or the food industry. In the United States, labels on beverage, food and dietary supplement must specify all ingredients, but the regulations do not require labeling the amount of caffeine in products (63).
Cardiovascular diseases are one of the main causes of death in the developed world countries (71). A prolonged QT interval has been associated with cardiovascular diseases such as TDP and SD. It is known that prolongation can be caused by certain drugs, but some recent studies also assume that QT prolongation might be caused by ingredients which can be found in ED (72).
In regulatory documents of FDA, EDs and other beverages are listed as dietary supplements; because of that, they are not strongly regulated and they do not have any limit to their caffeine levels.
In 1994, US congress enacted Dietary Supplement Health and Education Act (DSHEA) in which it says that herbal or other natural products should be classified as dietary supplements rather than food or drugs, and it does not place any limits on the amount of caffeine that can be

included in products according to the regulation that accepts herbal or other natural products (67).

Most ingredients in EDs are listed as Generally recognized as safe (GRAS). Exceptions are panax ginseng and taurine, which are not approved as GRAS, but because the taurine is an amino acid, it is considered to be used as a flavor by Flavors and Extracts Manufacturers Association of the United States (68).

What is really worrying is the fact that the most frequent users of EDs are children and young people. According to Substance Abuse and Mental Health Services Administration (SAMHSA) reports, the number of emergency room visits in US related to consuming EDs doubled from 10,068 visits in 2007 to 20,783 visits in 2011. Most of these patients were young people from 18-25 years (69).

Among the adolescents, mixing alcohol and EDs is not rare and can lead to life-threating diseases. Despite many actions of FDA and warnings that mixing alcohol and caffeine is not safe, such behavior continues to prevail among the adolescents.

Ingestion of up to 300 mg of caffeine can result in caffeine intoxication. In case of taking extreme doses of caffeine, it can also result in death. (70)

Children and young adolescents are not smaller version of adults. In 2011, Anais Fournier, a 14-year-old girl, died after drinking two 24-oz cans of Monster ED in a period of 24 hours. In 2013, Alex Morris, a 19-year-old boy, died after consuming two 16-oz cans of Monster ED. Both of them died because of the ED effects on the cardiovascular system (arrhythmia and cardiac arrest). 5-Hour ENERGY® drink is marketed as a dietary supplement. The Food and Drug Administration (FDA) requires the manufacturer, packer, and distributer to submit to the FDA all serious adverse event reports associated with the use of the dietary supplement in the United States. As of November 15, 2012, the Food and Drug Administration has received at least 90 patient reports, including more than 30 serious or life-threatening injuries, and 13 deaths identifying the use of 5-Hour ENERGY® drink in the report. These reports are currently under investigation by the FDA and have not been conclusively linked to the use of 5-Hour ENERGY® drinks.

The EDs should be labeled with exact amounts of their ingredients, as well as with a warning that they might cause some cardiovascular problems. FDA has the authority for changing the DSHEA and GRAS sections.

Most ingredients, such as Panax ginseng and Guarana that are found in EDs, are not for children and for now, not enough studies have been conducted to prove the opposite. Because the amounts of certain ingredients in EDs are much higher than their recommended therapeutic dosages, this is a last call to change the rules of labeling them.

It is obvious that EDs are becoming a bigger and bigger health issue day by day: not just because of the problems with cardiovascular diseases and obesity, but also due to significant costs to the health care system.
We agree with Dr. Marcie Schneider, the member of American Academy of Pediatrics (AAP), who said: "Energy drinks have no place in the diet of children and adolescents and it is necessary to educate doctors, parents and public about EDs" (73).

With this study, we suggested that we all should have become more informed about EDs effects on our health; this is especially true for the patients with QT-prolongation or other diagnosed heart disease.
There is a definite necessity for more studies in this field.

Limitations of the study

This study has some limitations. Firstly, the study population consisted of only healthy subjects that were younger than 40 years, based on the Framingham 10-year Risk for Heart disease calculation; age above 40 becomes positively associated with increased risk for heart disease.
Secondly, the sample size is maybe too small, but it was not easy to find volunteers for a 21-day-long investigative study that may include some serious ED-related adverse effects.
Thirdly, caffeine has a short half-life. We didn't perform ECG every day because the study was long. For participants, it is not practical to be coming every day. By performing an ECG every day, we would have lost some more participants. That is also the reason that we did not perform an ECG on day 2.
Another fact to be kept in mind is that our study was confined to healthy subjects with normal rhythm, no history of any cardiovascular disease, no concurrent use of any of the over- the-counter drugs and no use of any ED within one week before the start of the study. To the

exclusion criteria, we should maybe add non- habitual caffeine consumers (those who are not consuming coffee, tea or other caffeinated beverages more than one time per day), or ask the participants not to drink coffee or any other caffeinated product at least one week before the study.

6. CONCLUSION

All substances may be toxic after some dosage. Amounts of ingredients in EDs exceed recommended daily doses, in certain cases for more than 208 times (amount of vitamin B12 in 5-Hour ENERGY® drink). Product label of EDs do not list the exact amount of ingredients, especially caffeine content. On most labels, only ingredient amounts per serving are listed, which can also be tricky.

With our study, we did not conclude that 5-Hour ENERGY® drink is safe; we only established that it does not cause statistically significant differences on QTc if consumed within daily recommended dosages indicated on the 5-Hour ENERGY® drink bottle.

Our findings also showed some significant changes in blood pressure. Perhaps, they were only statistical and not clinical, but we should keep in mind that our volunteers were extremely healthy, and it is a question what could happen if a person with predispositions of cardiovascular diseases would drink the same amount of 5-Hour ENERGY® drink as our volunteers.
Once again, we assume that people who are older than 40, those who are hypertensive patients older or younger than 40 with treated or not treated hypertension are having higher risk for cardiovascular events after consuming EDs.

Until FDA does not start with appropriate role in regulation of EDs manufacturers, it will be difficult to expect that the general public will have a healthy and right attitude.
In accordance to that, more studies are needed.

Conflict of Interest

There is no potential conflict of interest or actual conflict of interest which includes financial, professional and personal affiliations.

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