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Evaluation the Toxicity of some Energy Drinks and its potential effects on the Athletes in IRAQ

Ahmed Sabah Qasim[1*], Nada Abdulalrahman Fleeh[1]

Biology Department, College of Science, Baghdad University, Baghdad, Iraq[1]

Corresponding author: 1*

ABSTRACT— This study was conducted to investigate the adverse effect of Energy Drinks (Red Bull and C4) taken by athletes in some blood and biochemical parameters which include lipids profile (HDL and LDL), Liver Function (AST, ALT, ALP, Bilirubin and Albumin), B. urea, Creatinine for kidney functions tests andHbA1C, ECG. The results indicated significant difference in biochemical parameters of athletes used EDs; there was a significant ($P < 0.05$) in the levels of Aspartate amino Transferase (AST), Alanine amino Transferase (ALT), Alkaline Phosphatase (ALP), Bilirubin and Albumin in athletes consumed deferent type of energy drinks (Red bull and C4) in different period of time when compared with control group. Also it was showed a significant ($P < 0.05$) in the levels of serum creatinine and blood urea compared with control group. Finally, the results also showed a significant ($P < 0.05$) in the mean of Low Density Lipoprotein (LDL) in experimental group when compared with control group. On other hand the results also showed a significant decrease ($P < 0.05$) in the mean of High Density Lipoprotein (HDL) in experimental group when compared with control group.

KEYWORDS: Energy drinks, Red Bull, C4, liver function tests, kidney function tests, lipid function tests, Athletes.

1. INTRODUCTION

Energy drinks have been defined in various terms all over the past years since their appearance in markets. Nevertheless, till now, no standard definition of an energy drink is documented in the scientific published literature. In the past, energy drinks are a group of beverages that has gained their fame since 1997 [1].

In other words, [2] defined energy drinks as beverages (for example Red Bull, C4, and Adrenaline Rush) that contain large doses of caffeine and other approved enhancers such as carbohydrates, taurine, glucuronolactone, niacin, inositol, and B-complex vitamins.

[3] reported that almost all energy drinks contain, in addition to caffeine, small concentration of nature-derived enhancers (guarana, ephedrine, yerba mate), also as all drinks products must contain simple sugars (glucose, fructose), also contain protein precursors as amino acids (taurine, carnitine, creatine), herbs (different types of Ginseng, Ginkgo biloba), maltodextrin, inositol, glucuronolactone (a naturally occurring sugar metabolite) and members of vitamins-B complex family as B6 and B12. More or less statement has been mentioned by [4].

Energy drinks are fortified beverages with added dietary supplements. Like most of the soft drinks in the market these drinks are aggressively marketed but are not always transparent in providing ingredient information and quantities on their labels. The promotion of natural ingredients in energy drinks to supply increased energy, increased alertness, and improved athletic performance leave a Physiologist wondering if

these drinks deliver what they claim) [5].

The topic of energy drinks is a matter of debate and, thus, true information about such drinks becomes a must. Energy or power drinks constitute a group of commercial products which contain a lot of ingredients that have the ability to generate energy; they are widely used, nowadays, all over the Arabic and International markets without any restrictions

Recently, we have noted appearance and growing of "generator of energy" concept among young and old people during exercise, under what is called energy drinks" that invaded Arabic and international markets, due to increment in the rate of its consumption. Scientific researchers noted that these drinks are attractive to the consumer's at all different ages, especially during the driving for long distances and during studying by all students during exam times; students consume a lot of these drinks in the belief that it can reduce the need for sleep, increase thinking ability and prolong time of studying [4].

Athletes believe that such drinks increase the body's energy which will be used during the exercise and supply them with extra power to can do extra sport and can built good musculature, and its ability to reduce fatigue while performing exercise.

All athletes turned to consumption of these products, rather than the past use of sport steroids injections, tonics, hormones especially growth hormones and proteins that may have side effects on the body, or may have been banned internationally or regulated illegal by the laws of most governments and athletes who may use these agents must be excluded or stopped. Also, most of the working women turned to consumption of these energetic drinks as a power source to enable them to can manage their work and perform their duties at home in a good manner.

These drinks have invaded both of the local and global markets under different brand names but with almost the same ingredients in different concentrations without any control from governments. Such drinks are not expensive, and thus accessible to everyone without any restrictions [6].

Various side effects were reported in relation with consumption of energy drinks, including death (Iyadurai and Chung 2007). In 2011, an Irish, 18 years old, athlete, dead because of playing a basketball game just after drinking 4 cans of the power drink Red-Bull (Alsunni 2011).

Health hazards associated with the uncontrolled consumption of power drinks are mainly attributed to their caffeine or caffeine-like ingredientcontents. Over dosage of caffeine might lead to extra systoles, palpitations, hypertension, anxiety, nausea, vomiting, hypocalcemia, metabolic acidosis, convulsions (World Health Organization, 2005), and, in scarce cases, death [7].

In addition, a higher risk of arterial hypertension (Brown et al., 2011) and insulin-nondependent diabetes mellitus [4] are documented, because excessive consumption of caffeine declines insulin sensitivity (Lee et al., 2005). Excessive caffeine consumption during pregnancy increases the risk of late miscarriages, smaller-than-normal for gestational age babies and stillbirths [8].

Although these ingredients are responsible for the desired effects of energy drinks (like increasing the level of energy, enhancement of physical activity, reduction of mental exhaustion and improvement in the mood), but they can also cause hazardous effects. Consumption of caffeinated energy drink may induce nephrotoxicity, hematological disorders, hepatitis and pancreatitis. Furthermore, the high sugar content

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results in obesity and diabetes (Khayyat et al., 2014; [6]. While it is known that acute overdose of these products has been associated with toxicity, there is growing concern that chronic use of energy drinks and dietary supplements may cause neurologic and cardiovascular toxicity [9].

2. Material and methods

Ninety athletes age (20-35) were participated in this study with the cooperation's of collage physical educations university of Baghdad and distributed for three groups, as follow

1. Group A: control group 30 Athletes without use any energy drinks.
2. Group B: 30 athletes who used energy drinks (Red bull).
3. Group C: 30 athletes who used energy drinks (C4).

2.2.1 Sample Collection from Iraqi Markets

Choosing two types of energy drinks (Red Bull, C4) available in the local markets of Baghdad and consumed by athletes. Table (2-4).

2.2.2 Sample Collections for serological analysis

After collecting general information from Athletes according to questionnaire Five milliliters (5 ml) venous blood was obtained from the Athletes. All blood samples were centrifuged then stored at -20°C until assayed for laboratory investigations (Rafiean and Hamid, 2013).

Levels of serum SAST, SALT, urea, creatinine, Albumin, bilirubin, ALP, LDL and HDL were measured using standard kits [10].

2.2.3 Sample Preparation

(1) The specimen were store at -20C until analysis.
(2) Each specimen was dispense into an analyzer cup in the appropriately barcoded analyzer tube.
(3) All barcoded tubes were placed on the Hitachi sample wheel starting at position with the barcodes facing center. Ensure that the instrument is calibrated and verified before starting the unknown sample analysis [10].

Table 1: Energy Drinks samples used in this study

Common name	Name
Red Bull	*Red Bull*
C4	Cellucor launched

Biochemical Parameters

Measurements of Liver Function tests

Venous blood was collected (5 ml) for testing in the morning on an empty stomach and transferred to the laboratory within 1 hour of collection. All assays were done on the same day. Blood samples for biochemical measurements were collected into tubes containing coagulation accelerator and serum separator. In order to obtain the serum for testing, blood samples were centrifuged at 2000g for 10 minutes. The measurements of total bilirubin (BIT), alkaline phosphatase (ALP), alanine aminotransferase (ALT),

Aspartate aminotransferase activity (AST) and Albumin were achieved by spectrophotometric methods with a Pentra 400 Horiba biochemical analyser (France), using original manufacturer reagent kits [11].

Kidney Function tests

Measurements serum Urea concentration

The enzymatic method is used to measure the level of urea in the serum by using several tools Measurement Kits, ready-made from the French company AS Bio Merieux, where the enzyme urease hydrolyses urea to Ammonia and carbon dioxide according to the following equation:

Urea+H2OUrease→ 2NH3+CO2

Ammonium ions react in the alkaline medium with salicylate and hypochlorite

Hypochlorite gives the indophenol compound green in color and stimulates this reaction by adding Sodium Nitroprusside according to the following equation:

NH+4+ + salicylate + hypochloriteNitroprusside→ indophenol

There is also a direct relationship between serum urea concentration and color intensity [12].

Measurements serum Creatinine concentration

Blood samples were withdrawn by venipuncture from the antecubital vein of the Athletes before intensive training. The blood was allowed to clot at room temperature and centrifuged at 2000g for 10 minutes to obtain serum. Serum creatinine by the modified Jaffe method as adapted for the autoanalyser by Synchron CX systems (Beckman Instruments) by using special kit [13].

Lipid profile Tests

Measurements of high-density lipoprotein (HDL) Concentration

The method [14] was used to estimate the level of high-density lipoprotein (HDL) in Serum by enzymatic method and using phosphotungstic acid and magnesium ion, where LDL, chylomicrons and low protein are separated. The density is very VLDL and therefore gets a filtrate containing HDL by the process Centrifugation, where the cholesterol bound to it is estimated by enzymatic solution and using a measuring kit Ready-made Kits, produced by the English Randox Company.

Measurement of Serum Low Density Lipoprotein (LDL-C):

The concentration low density lipoprotein cholesterol (LDL-C) in serum were estimated by using diagnostic kits (Pars Azmoon kit, IRI) with an automatic analyzer (Abbott, model Alcyon 300) [15].

3. Results and Discussions

Kidney Function Tests

Table (2): Results of urea and Creatinine tests in athletes consumed Energy Drinks

Mean±SE			
Durationof ED use	**B.Urea (mg/dl)**	**S.Creatinine (mg/dl)**	**Type of EDs**
Control	**(22.80±0.77)**	**(0.80±0.03)**	**--------**
More than 12<months	**38.47±1.32**	**1.40± 0.06**	**Red bull**
6-12 months	**29.89±1.26**	**1.30±0.12**	**Red bull**

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3-6 months	**28.20±0.88**	**1.12±0.10**	**Red bull**
More than 12<months	**38.56±0.65**	**1.6216±0.13**	**C4**
6-12 months	**32.41±1.26**	**1.25±0.03**	**C4**
3-6 months	**27.85±0.35**	**1.11±0.02**	**C4**
LSD	**3.97**	**1.06**	--------
(P≤ 0.05			

Urea: Athletes who consumed EDs for a period from 3-6 months, the level of serum urea in Athletes consumed Red Bull was (**28.20±0.88)** mg/dl and t consumed C4 was (**27.85±0.35)** mg/dl. While, Athletes who consumed EDs for a period 6-12 months, the level of serum urea consumed Red Bull was (**29.89±1.26)** mg/dl and consumed C4 was (**32.41±1.26)** mg/dl**.** Finally, Athletes who consumed EDs for a period more than one year the level of serum urea consumed Red Bull was (**38.47±1.32)** mg/dl and consumed C4 was (**38.56±0.65)** mg/dl**,** respectively as compared with the control (**22.80±0.77)** mg/dl.

LSD value of urea serum was (3.97), show significant differences (P≤0.05) between results of urea in different period of time and different type of EDs when compare with control.

Creatinine: Athletes who consumed EDs for a period from 3-6 months, the level of serum Creatinine in Athletes consumed Red Bull was (1.12±0.10) mg/dl and consumed C4 was (1.11±0.02) mg/dl. While, Athletes who consumed EDs for a period 6-12 months, the level of serum Creatinine consumed Red Bull was (1.30±0.12) mg/dl consumed C4 was (1.25±0.03) mg/dl. Finally, Athletes who consumed EDs for a period more than one year the level of serum Creatinine consumed Red Bull was (1.40± 0.06) mg/dl and consumed C4 was (1.6216±0.13) mg/dl, respectively as compared with the control (0.80±0.03) mg/dl.

LSD value of serum Creatinine was (1.06), show significant differences (P≤0.05) between results of urea in different period of time and different type of EDs when compare with control.

Tests for measuring creatinine and blood urea are basic indicators that are used to diagnose renal function to determine renal damage, as urea represents the final product of protein metabolism (exogenous and endogenous), while creatinine is produced from muscle creatine and is the result of distinguishing Edelstein (Phosphocreatine, 2008). Significant compared to the control group, which means that there is a reduction in renal function, which leads to keeping water and salts in the body leading to edema and thus reducing the volume of daily urine [17]. It is known that the renal glomerulus is selectively permeable, and that permeability depends on the size and charge These molecules, damage to the glomerulus, can reduce this selectivity and thus cause the escape of important molecules such as immunoglobulins, albumin, erythrocytes, etc. (Delimaris, 2013).

The occurrence of an acute inflammatory response may lead to tissue damage resulting from the activation of the complement system and the migration of platelets, macrophages and cells. The complement system is the major influencer of humoral immunity [16]. The results of this study agree with what was stated by Loll et al. (2011).

The results in this study was confirmed by several authors [17], Abd El-Moneim et al., 2009) who showed

that caffeine can elevate creatinine and urea concentration in the blood serum.

Liver Function Tests

Table 3: Results of Liver Functions Tests (Bilirubin, ALP, ALT, AST and Albumin) in athletes consumed Energy Drinks

Mean ±SE						
Durationof ED use	Bilrubin(mg/dl)	ALP U/L	ALT U/L	AST U/L	Albumin mg\l	Type of EDs
Control	**0.47±0.02**	**42.51±1.03**	**28.44±0.95**	**31.11±0.73**	**3.63±0.07**	------
More than 12<months	**0.89±0.04**	**81.02±3.18**	**39.07±0.63**	**38.16±0.87**	**4.94±0.08**	**Red bull**
6-12 months	**0.87±0.04**	**77.71±3.62**	**38.11±1.05**	**37.09±0.46**	**4.52±0.08**	**Red bull**
3-6 months	**0.75±0.05**	**72.97±2.84**	**36.83±0.74**	**36.69±0.74**	**4.41±0.29**	**Red bull**
More than 12<months	**1.11±0.05**	**91.19±3.45**	**41.04±1.18**	**39.06±0.34**	**5.02±0.12**	**C4**
6-12 months	**1.06±0.04**	**82.59±4.59**	**39.84±0.98**	**37.86±0.67**	**4.65±0.17**	**C4**
3-6 months	**0.88±0.05**	**77.08±2.36**	**38.58±1.13**	**36.83±0.66**	**4.59±0.08**	**C4**
LSD	**0.96**	**2.78**	**2.33**	**1.85**	**1.27**	----
(P≤ 0.05)						

Bilirubin: Athletes who consumed EDs for a period from 3-6 months the level of Bilirubin in Athletes consumed Red bull was (0.75±0.054)mg\l and consumed C4 was (0.88±0.05) mg\l. While, Athletes who consumed EDs for a period from 6-12 months, the level of Bilirubin in Athletes consumed Red bull was (0.87±0.04)mg\l and consumed C4 was (1.06±0.046) mg\l. Finally, Athletes who consumed EDs for a period more than one year the level of Bilirubin in Athletes consumed Red bull was (0.89±0.044)mg\l and consumed C4 was (1.11±0.05)mg\l as compared with the control (0.47±0.02)mg\l.

LSD value of Bilirubin was (0.96), show significant differences (P≤0.05) between results of Bilirubin in different period of time and different type of EDs when compare with control.

Albumin: Athletes who consumed EDs for a period from 3-6 months the level of Albumin in Athletes consumed Red bull was (4.41±0.29)mg\l and consumed C4 was (4.59±0.08)g\dl While, Athletes who consumed EDs for a period from 6-12 months, the level of Albumin in Athletes consumed Red bull was (4.52±0.08)g\dl and consumed C4 was (4.65±0.17)g\dl. Finally, Athletes who consumed EDs for a period more than one year the level of Albumin in Athletes consumed Red bull was (4.94±0.08)g\dl and consumed C4 was (5.02±0.12)g\dl as compared with the control (3.63±0.07) g\dl.

LSD value of Albumin was (1.27), show significant differences (P≤0.05) between results of Albumin in different period of time and different type of EDs when compare with control.

Aspartate aminotransferase (AST): Athletes who consumed EDs for a period from 3-6 months the level of

AST in Athletes consumed Red bull was (36.69±0.74)U/L and consumed C4 was (36.83±0.66)U/L While, Athletes who consumed EDs for a period from 6-12 months, the level of AST in Athletes consumed Red bull was (37.09±0.46)U/L and consumed C4 was (37.86±0.67)U/L. Finally, Athletes who consumed EDs for a period more than one year the level of AST in Athletes consumed Red bull was (38.16±0.87)U/L and consumed C4 was (39.06±0.34)U/L as compared with the control (31.11±0.73)U/L.

LSD value of AST was (1.85), show significant differences (P≤0.05) between results of AST in different period of time and different type of EDs when compare with control.

Alanine aminotransferase (ALT): Athletes who consumed EDs for a period from 3-6 months the level of ALT in Athletes consumed Red bull was (36.83±0.74) U/L and consumed C4 was (38.58±1.13)U/L While, Athletes who consumed EDs for a period from 6-12 months, the level of ALT in Athletes consumed Red bull was (38.11±1.05) U/L and consumed C4 was (39.84±0.98) U/L. Finally, Athletes who consumed EDs for a period more than one year the level of ALT in Athletes consumed Red bull was (39.07±0.63)U/L and consumed C4 was (41.04±1.18) U/L as compared with the control (28.44±0.95) U/L.

LSD value of ALT was (2.33), show significant differences (P≤0.05) between results of ALT in different period of time and different type of EDs when compare with control.

Alkaline phosphatase (ALP): Athletes who consumed EDs for a period from 3-6 months the level of ALP in Athletes consumed Red bull was (72.97±2.84) U/L and consumed C4 was (77.08±2.36) U/L While, Athletes who consumed EDs for a period from 6-12 months, the level of ALP in Athletes consumed Red bull was (77.71±3.62) U/L and consumed C4 was (82.59±4.59) U/L. Finally, Athletes who consumed EDs for a period more than one year the level of ALP in Athletes consumed Red bull was (81.02±3.18)U/L and consumed C4 was (91.19±3.45) U/L as compared with the control (42.51±1.03) U/L.

LSD value of ALP was (2.78), show significant differences (P≤0.05) between results of ALP in different period of time and different type of EDs when compare with control.

The increase in the levels of AST, ALT, ALP, and total bilirubin rate indicates a defect in liver metabolism [18], it was found that the increase in the levels of AST, ALT, ALP indicates the susceptibility of some compounds to stimulate the Oxidative Stress of the liver releasing large amounts of free stems that affect the composition and function of the cell [19]. Free radicals that affect the structure and function of the cell as well as the physiological concentrations of free radicals are important, and when the levels of free radicals rise, it leads to inflammation, arthritis, and kidney inflammation, and leads to various Diseases such as cancer, high pressure, diabetes and atherosclerosis(Haque et al. 2015). Energy drinks contain many additives including caffeine, taurine, B-vitamins, and other ingredients, Niacin (vitamin B3) has been shown to cause hepatotoxicity ranging from mild elevations in the aminotransferases to fulminant hepatic failure, and available data suggest a greater than 50% chance of hepatotoxicity when doses of niacin exceed 2,000 mg/day (McKenney et al., 1994).

Vivekanandarajah et al., (2011) described a young who drank 10 cans of energy drink over 2 weeks, resulting in acute hepatitis she had consumed 300 mg per day of niacin, and they concluded this was most likely the cause of her acute hepatitis.

Results showed a significant icreased in the concentration of alanine aminotransferase and aspartate aminotransferase in all energy drinking groups compared to the control group. This result is differing from

results of Alrasheedi and Abdel-Mageid (2007).

Lipid Profile

Table (4): LDL and HDL in athletes consumed Energy Drinks

Mean±SE			
Durationof ED use	**HDL mg\l**	**LDL mg\l**	**Type of EDs**
Control	**50.07±1.24**	**80.34±3.15**	**--------**
More than 12<months	**43.60±1.813**	**121.68±6.24**	**Red bull**
6-12 months	**47.50±1.76**	**119.23±8.34**	**Red bull**
3-6 months	**47.01±1.58**	**106.68±6.23**	**Red bull**
More than 12<months	**40.45±0.90**	**131.82±3.39**	**C4**
6-12 months	**42.85±0.98**	**128.01±6.04**	**C4**
3-6 months	**42.34±1.22**	**107.07±4.49**	**C4**
LSD	**3.03**	**4.52**	
(P≤ 0.05			

High density lipoprotein (HDL): Athletes who consumed EDs for a period from 3-6 months the level of HDL in Athletes consumed Red bull was (47.01±1.58) mg\l and consumed C4 was (42.34±1.22) mg\l While, Athletes who consumed EDs for a period from 6-12 months, the level of HDL in Athletes consumed Red bull was (47.51±1.76) mg\l and consumed C4 was (42.85±0.98) mg\l. Finally, Athletes who consumed EDs for a period more than one year the level of HDL in Athletes consumed Red bull was (43.61±1.81) mg\l and consumed C4 was (40.45±0.91) mg\l as compared with the control (50.07±1.24)mg\l.

LSD value of HDL was (3.03), show significant differences (P≤0.05) between results of HDL in different period of time and different type of EDs when compare with control.

Low density lipoprotein (LDL): Athletes who consumed EDs for a period from 3-6 months the level of HDL in Athletes consumed Red bull was (106.68±6.23) mg\l and consumed C4 was (107.07±4.49) mg\l While, Athletes who consumed EDs for a period from 6-12 months, the level of HDL in Athletes consumed Red bull was (119.23±8.34) mg\l and consumed C4 was (128.01±6.04) mg\l. Finally, Athletes who consumed EDs for a period more than one year the level of HDL in Athletes consumed Red bull was (121.684±6.241985036) mg\l and consumed C4 was (131.82±3.39) mg\l as compared with the control (80.34±3.15)mg\l.

LSD value of HDL was (4.52), show significant differences (P≤0.05) between results of HDL in different period of time and different type of EDs when compare with control.

It was shown that four days long diet with high content of sucrose (glucose and fructose) results in increased de novo lipogenesis by 200-300 per cent, other studies using high fructose concentrations (up to 30 per cent of total calories) revealed elongation of lipidemia period [20]. The scientific literature contains numerous evidences suggesting simple sugars, such as glucose and fructose, may promote the development of insulin resistance, lipid peroxidation and liver tissue inflammation, as well as may act as end products of glycosylation [21]. As the main ingredient of energy drinks, which actually provided a tonic effect, caffeine

affects the metabolism of carbohydrates and lipids in the liver. Caffeine facilitates glucose uptake by hepatocytes and deposition of glycogen in the liver because of increased blood glucose level after consumption of energy drink [22], as well as induces fatty acid oxidation by activating autophagy of intracellular lipids in the liver (Sinha et al., 2014).

The study has agree with Barter, (2011) the decrease in the cholesterol concentration induced by Red Bull might have been due to the elevated content of taurine and/or niacin present in the ED. This change is somewhat expected because both taurine and niacin are used in the prevention and cure of atherosclerosis More precisely, taurine reduces serum cholesterol and niacin reduces serum cholesterol and triglycerides and decrease HDL concentration, this effect can in turn be a cause of the myocardial dysfunctions reported in the chronic consumption of EDs. A role of cholesterol is to stiffen the cellular membranes and maintain the shape of cells by forming “bridges” (lipid rafts) in the regions where the membrane proteins are expressed [23]. Additionally, cholesterol controls the membrane fluidity, and, consequently, plays an important role in the the cholesterol to phospholipid ratio [24].

4. Conclusion

Biochemical parameters considered an important biomarker gives sign for toxicity; consumption of energy drinks by athletes has clear effects on the biochemical parameters, which are first indicators of toxicity.

The different type of energy drinks (Red Bull and C4) causes significant alteration in biochemical parameters of the liver, kidney, and Lipid profile, while the consumption of energy drink (C4) by athletes showed clear effects on the biochemical parameters (liver, kidney and lipid profile) more than Energy Drink (Red bull). Also the athletes consumed Energy drinks (Red bull and C4) more than 12 months has more clear change in biochemical parameters (liver, kidney, lipid and Blood sugar) than athletes consumed the energy drinks less than 12 months.

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