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Short term consumption for two types of energy drink on physiological parameters in young rats

A graduated project submitted to the department of pharmacology and toxicology, the committee of projects discussing of the College of Pharmacy, University of Basrah

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Abstract

The current work aimed at studying the adverse effects of two popular kinds of energy drinks (Red Bull and monster) in short period on some physiological parameters in young rats. Healthy male Wister albino rats (eight weeks old. The animals were divided into 3 groups of (N=6). Animals of the first group were kept on normal diet and water and served as control. While animals in the other two groups were orally administered by gavage for two weeks with a single daily dose (1.5 ml/100g b.w) of Red Bull and Monster respectively. The hematological profile results of the treated rats in comparison with the control showed abnormalities and elevation in the white blood cells parameters for monster energy drink. The values of lymphocytes was elevated in Red bull energy drink compared to control and other type of drink . Whereas granulocytes and monocytes were increased in both groups of energy drink. In contrast, the values of Red blood parameters , RBCs indices and platelets count displayed non-significant differences . The concentrations of thyroid stimulating hormone was significantly reduced in both treated groups with energy drink, while the concentration of Triiodothyronine was significantly increased ,Thyroxin and Luteinizing hormone revealed non-significant differences. Finally , Follicle stimulating hormone and Prolactin hormone were significantly increased in both energy drink groups. , Interleukin 6 levels is significantly increased in both groups of energy drink compared to the control. While C-reactive protein level in the same table revealed significant increased only in Red bull group compared control and other energy drink group. The histopathological study on liver and kidney of treated groups showed many changes such as inflammatory cells infiltration and necrosis and hypertrophy. So, we can be conclude the short term of energy drinks consuming effect histopathologically and physiologically on kidney and liver of young rat because of caffeine or combination of caffeine with taurine or with sugar toxicity which depend on its concentration and period of consuming.

1.Introduction

First appearance of energy drinks (EDs) was in Europe and Asia in the 1960 as a result of customer requirements for dietary supplements that give energy [1]. Many Saudi studies found that more than half of the consumers were young (13 - 35 years old), more than half consumed it for over a year, and over 40 % used to drink more than 3 cans per week [2]. Centers for Disease Control and Prevention reported that high school students consume EDs almost at the same rate as they consume soda [1]. Indeed, the rate of ED consumption might be higher than estimated levels in this self-reporting survey, since such surveys usually have high probability of underestimation. It has been revealed that EDs contain mainly taurine, glucuronolactone, caffeine, ginseng and guarana [3]. These substances, most of which act as stimulants, are not included in the list of materials under regulation by the Food and Drug Administration (FDA) of the United States of America. The levels of these stimulants vary amongst different brands of EDs, and in most cases, are higher than values allowable [4]. A study has shown that the caffeine levels in EDs are between 50 and 505 mg/ can, which are much higher than the caffeine content of one can of Coke (34 mg) [5]. Reports of significant, adverse health problems due to ingestion of EDs have increased in recent years. Indeed, in 2013, ED-associated emergency interventions by the US Substance Abuse and Mental Health Services Administration doubled from 10,068 in 2017 to over 20,000 in 2011 [6]. A major constraint in understanding the link between EDs and the adverse effects of their consumption is that very little is known about the toxicity of the various compounds present in them. However, based on reported cases of ED-associated health problems, and the well-established physiological effects of the active ingredients of EDs, it is very likely that the observed adverse effects of EDs are linked to their compositions [3]. A significant increase in the incidence of male infertility has been described in the literature worldwide, which generates questions about its causes. There are several substances present in our daily lives that exhibit potential interferences with biological functions, such as reproduction, embryonic development, growth, and metabolism [7]. For this reason, these substances are more frequently becoming the focus of research with the objective of observing their effects in the long term and their consequences for humans, even at very low concentrations [8].

Energy drinks mostly contain caffeine, taurine, l-carnitine, carbohydrates, glucuronolactone, vitamins and other herbal supplements like ginseng and guarana [9]. Majority of these energy drinks are targeted at athletes, teenagers and college students who consume large doses of these drinks in the hope to increase their energy level or compensate lack of sleep [10]. Research suggests that energy drink formulations, in addition to increasing energy utilization, may also improve mood, enhance physical endurance, reduce mental fatigue and increase reaction time

[11]. Beck et al. (2006) reported that caffeine-containing supplement may be an effective supplement for increasing upper body strength and therefore could be useful for competitive and recreational athletes who perform resistance training [12] . According to the manufactures , the stimulating effects of these drinks are due to interaction between various ingredients [13]. Despite their potential beneficial effects, massive consumption of energy drinks result in life-threatening toxicity. Additives such as guarana, yerba mate, cocoa and kola nut may increase the caffeine content of energy drinks [14]. Reissig et al. (2009) mentioned that different brands of energy drinks contain caffeine ranging from 50 mg to 550 mg per can or bottle [15] . On the other hand, Kavita et al. (2008) reported that, caffeine content of these products is presently unregulated and rapid growth in the consumption of these supplements has resulted in increasing reports of caffeine poisoning [16]. Several warnings have been issued regarding the potential adverse effects of energy drinks including hepatotoxicity and nephrotoxicity [17], neurologic complications [18], alterations in the cardiovascular system [19] and changes in the structure and function of secretory glands [20]. The current work aimed at studying the adverse effects of two popular kinds of energy drinks (Red Bull and monster) in short period on some physiological parameters in young rats.

2. Materials And Methods

2.1. Animal care

Eighteen healthy male Wister albino rats (eight weeks old) weighing 60 g–95 g were purchased from animal center in College of Veterinary/University of Basrah (Basrah, Iraq). The animals were housed in animal facility at College of Pharmacy, University of Basrah. Rats were kept in a 12 h light/dark environment at a constant temperature of 25 ± 1 °C with a relative humidity of 55 ± 5%. All animals were used for in vivo experiments and were approved by the Animal Research Ethical Committee of Basrah University

2.2. Experimental design

The energy drinks Red Bull and Monster were bought from local market at Basrah city ,Iraq. The major constituents of these energy drinks are caffeine, guarana, taurine, ginseng, vitamins and carbohydrates [21]. The animals were divided into 3 groups of 6 animals each . Animals of the first group were kept on normal diet and water and served as control. While animals in the other two groups were orally administered for two weeks with a single daily dose (1.5 ml/100g b.w) of Red Bull and Monster respectively. After two weeks, rats from both control and experimental groups were anaesthetized with chloroform. (Sigma, MO) and decapitated. The

peripheral blood samples were collected via cardiac puncture method into blood collecting tubes containing few crystals of EDTA as an anticoagulant

2.3. Hematological analysis

At the end of experiment , animals were weighed and anesthetized using chloroform. Blood samples were collected from the rats by cardiac puncture then they were transferred into a lavender top collection tube containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) and used for hematological analysis. Analysis of complete blood count (CBC) was performed through automated blood cell analyzer (NP-26H,NIPIGON, Canada).The parameters analyzed were total white blood cell (WBC) count, lymphocyte and monocyte counts, red blood cells (RBC) count, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT), mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW). The automated blood cell analyzer was used for evaluation the blood parameters.

2.4. Biochemical analysis

2.4.1. Evaluation of hormones

Blood samples were centrifuged (2400 rpm, 20 min) using a refrigerated apparatus (Genex, Florida, USA) to obtain serum. Thyroid stimulating hormone, Triiodothyronine ,Thyroxine ,Follicle stimulating hormone , Prolactin and Luteinizing hormone levels in the serum were evaluated by commercial kits and I-Chroma II Immunoassay Analyzer (Boditech, Korea).

2.4.2. **Evaluation of Inflammatory markers**

The common markers used to assess the inflammatory response in rat are IL-6, C-reactive protein (CRP) , To esstimate the level of each previous test , 75 µL of serum was taken for each blood sample , then they were assessed by commercial kits (Fine care IL-6 , Fine care CRP) and Finecare FIA Meter Plus Analyzer (Boditech, Korea).

2.5. Histopathological Evaluation

After euthanasia, the whole kidneys , livers , brains , thyroid glands and pancreases were carefully removed and they were fixed with 10% neutral formalin. The organs of rats were dissected, embedded in paraffin and 5µm sections were cut by using a rotary microtome and the samples were then stained with haematoxylin and eosin (H&E) for microscopic examination (Mescher, 2015).

2.6. Statistical analysis

All results such as changes in animal body weight, hematology and biochemistry studies were analyzed with the software Graph Pad Prism 5 for windows (San Diego, CA, USA). Findings were reported as Mean ± Standard Error of Mean (SEM). One-way analysis of variance (ANOVA) was performed followed by Bonferroni's multiple comparison tests (MCT).

3. Results

3.1. Hematological evaluation

As shown in table (1), the hematological profile of the treated rats in comparison with the control showed abnormalities and significant increase ($p<0.002$) in the white blood cells parameters for monster energy drink. The values of lymphocytes was elevated in Red bull energy drink compared to control and other type of drink . Whereas granulocytes and monocytes (MID) were significantly increased ($p<0.001$) in both groups of energy drink. In contrast, the values of Red blood parameters , RBCs indices and platelets count displayed non-significant differences .

Table (1): Effect of energy drinks on blood parameters (Mean±SE), (N=6)

	Control	Red bull	Monster	LSD
WBC	6.488±1.68	6.566 ±0.76	11.656±0.97**	1.112057
LYM%	86.58±1.57	75.52 ±0.76**	84.86±3.66	4.194201
GRAN%	4.96±1.32	12.38±2.4**	9.34±3.55**	3.571127
MID%	8.78±0.75	15.08 ±2.2**	14.1±2.43**	2.681976
RBC	5.966±0.28	4.816 ±1.30	4.686±1.19	NS
HGB	12.96±0.59	11.04 ±2.76	12.6±1.07	NS
HCT	34.88±0.71	29.76 ±8.03	36.24±0.83	NS
MCV	57.74±0.92	57.96 ±1.61	58.24±0.95	NS
MCH	21.98±1.06	21.84 ±1.24	22.5±1.5	NS
MCHC	37.68±0.79	36.56 ±2.69	37.24±1.93	NS
PLT	923.6±84.85	688 ±430.61	903.6±395.86	NS

3.2. Biochemical evaluation

3.2.1. Effect of energy drinks on hormones

According to the results shown in table (2) , the concentrations of thyroid stimulating hormone (TSH) was significantly reduced in both treated groups with energy drink, while the concentration of Triiodothyronine was significantly increased ($p<0.02$), Thyroxine and Luteinizing hormone revealed non-significant differences. Finally , Follicle stimulating hormone (FSH) and Prolactin hormone were significantly increased ($p<0.01$) in both energy drink groups.

Table (2): Effect of energy drinks on hormones (Mean±SE), (N=6)

Hormones	Control	Red bull	Monster	LSD
TSH (mlu/L)	2.238±0.337	0.768±0.11**	1.386±0.34*	0.413033
T3 (nmol/L)	4.838±0.75	7.886±1.26*	7.462±2.38*	2.228081
T4 (nmol/L)	111.204±18.31	96.752±4.52	103.95±4.27	NS
FSH (mlU/ml)	2.464±0.38	4.56±0.34**	4.49±0.28**	0.467899
LH (mlU/ml)	1.746±0.31	1.78±0.34	1.606±0.12	NS
PROLACTIN (ng/ml)	1.962±0.34	4.226±0.65**	5.524±0.29**	0.632346

3.2.3. Effect of energy drinks on inflammatory markers

Depending on the results in table (3) , Interleukin 6 (IL-6) levels is significantly increased ($p < 0.05$) in both groups of energy drink compared to the control. While C-reactive protein (CRP) level in the same table revealed significant increased ($p < 0.003$) only in Red bull group compared control and other energy drink group.

Table (3): Effect of energy drinks on inflammatory markers (Mean±SE), (N=6)

Inflammatory markers	Control	Red bull	Monster	LSD
IL-6 (pg/ml)	86.092± 3.55	105.386±3.82**	170.902±16.67**	13.90299
rapid CRP (mg/L)	4.56±0.55	5.76±0.167*	4.8±0.54	0.63448

3.3. Organs histopathological evaluation

Histopathological examination of the liver in control group (Fig. 1A) showed intact hepatocytes architecture that were while evident in rats fed olive oil enriched diet shown slightly loss normal structure of hepatocytes (Fig. 1B). While several morphological alterations were observed in rats treated with Red bull energy drink . These alterations were manifested, the hepatic vein was markedly dilated and congested with blood , also, the hepatic lobe depicted irregular localization of hepatocyte (black star). Moreover, Monster energy drink treated group displayed many of hypertrophy between hepatocytes and irregular structure of hepatocytes as shown in (Fig. 1C) .

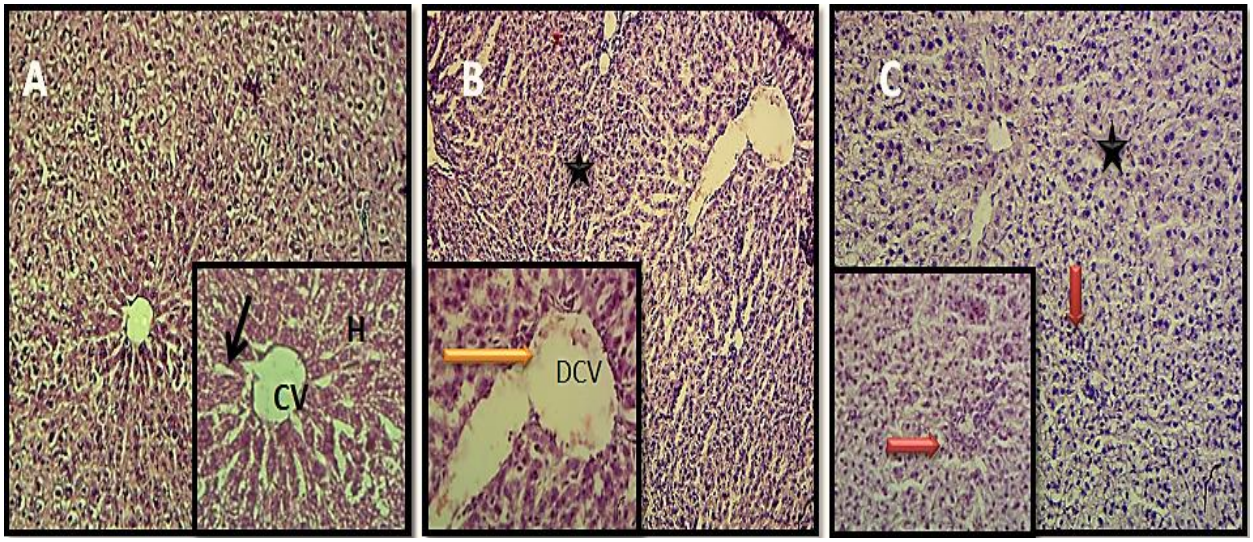

Figure 1. Photomicrographs of the liver tissue in albino rats. *(A) Control group displayed normal liver histology: normal structure of hepatocytes (H) , and normal central vein (cv) also normal sinusoid (S.) (B) Red Bull energy drink treated group revealed loss normal structure of hepatocytes (black star) also dilated in central vein (DCV) and congestive central vein (orange arrow) . (C) Monster energy drink treated group displayed many of hypertrophy between hepatocytes(red arrow). Using H&E stain and magnifications 100x and 400x for main and inset images respectively.*

the kidney tissue of rats in control group showed normal glomeruli, tubules and vessels both proximal and distal tubules with very little interstitial between the tubular structures in the cortex (Fig. 2A). also normal cellularity of the glomeruli and normal capillary walls thickness were observed in control group . However, light microscopic evaluations of kidney sections from rats that given an energy drink (Red bull group) also (Monster group) detected bleeding and congestion in the blood vessels and dilatation in glomerular capillaries (Fig. 2C and 2Dt). Moreover, the Bowman's space was enlarged and several tubular structures were defected in experimental group compared with control group .

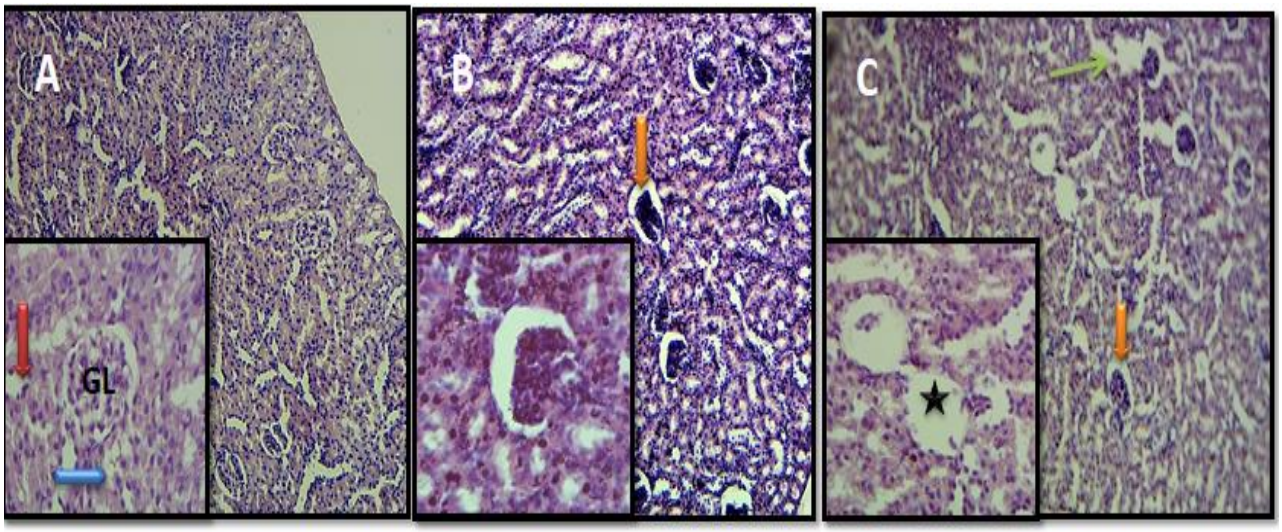

Figure 2. Photomicrographs of the kidney tissue in albino rats. *(A) Control group showed normal kidney histology: normal glomerulus (GL), proximal (red arrow) and distal tubules (blue arrow). (B) Red bull energy drink treated group revealed glomerulus shrinkage (orang arrow) and Bowman's space enlargement .(C) Monster energy drink treated group revealed tubular necrosis(green arrow), also, showed glomerulus shrinkage (orange arrow),Bowman's space enlargement and glomerulus necrosis (black star) . Using H&E stain and magnifications 100x and 400x for main and inset images respectively.*

4. Discussion

Berger and Alford (2009) reported that the combination of caffeine and taurine, which are some components of energy drinks, excessive ingestion can produce ischaemia of myocardial by inducing coronary vasospasm (22). On the other hand, it is well established that taurine is associated with bile acids and helped fat digest (23). Our light microscopic results revealed leucocytes infiltration through the hepatocytes. This might be due to different reaction of taurine associated with other active ingredients of the energy drinks as caffeine. Khayyat and Mubarak (2012) studies showed that the cytoplasm of rats' hepatic cells, which consumed energy drinks, appeared vacuolized with presence of lipid droplets. s. These could be attributed to degenerative changes within the liver cells (24). On the other hand, many works studied the ultrastructural alterations of hepatocyts of animals that consumed energy drinks and reported presence dilatation and fragmentation of rough endoplasmic reticulum cisternae , which can damage hepatocytes (25). Treatment with Monster revealed significant increase in total WBC count at the 2nd week, while all the other parameters of WBCs were also affected such as LYM % ,group red bull energy drink display significant increase , moreover , granulocytes shown elevated in its level , this result not accepted with result of Bassini-Cameron et al. (2007), in animals treated with Power Horse, neutrophils were significantly decreased, while lymphocytes were markedly elevated. Marked changes of lymphocytes and monocytes were recorded after 4 weeks of treatment with Code Red . Elevation of WBC count might indicate activation of immune system, a normal cell–mediated immune response and increase in lymphocytes and monocytes could be attributed to the action of caffeine that stimulates hemopoietic system to release more of these cells demonstrated that caffeine treatment during exercise leads to a greater degree of leukocytosis, lymphocytosis and muscle damage (26). the concentrations of thyroid stimulating hormone (TSH) was reduced in both treated groups with energy drink, while the concentration of Triiodothyronine was significantly increased , Thyroxine and Luteinizing hormone revealed non-significant differences. Finally , Follicle stimulating hormone (FSH) and Prolactin hormone were significantly increased in both energy drink groups. energy drink effect directly on hypothalamus region also effect on thyroid gland

5. Conclusions

short term of energy drinks consuming effect histopathologically and physiologically on kidney and liver of young rat because of caffeine or combination of caffeine with taurine or with sugar toxicity which depend on its concentration and period of consuming

6. References

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