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Evaluation of the physiological and histological effects induced by some energy drinks on male rabbits

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Energy drinks are widely available and popular. This raised concerns about their effects on human health. The present study aimed to evaluate the physiological and histological effects induced by the following energy drinks: Power Horse, Bison and Code Red, on male rabbits. Twenty male rabbits were divided into four groups (5 rabbits each). Group 1 was served as control group, while groups 2, 3, and 4 were daily ingested with only one type of energy drinks for 5 weeks. Rabbits were weighed before and during the experiment. After five weeks, samples were taken from animals of each group as follows: Whole blood and biopsy from liver were collected for biochemical and histopathological examinations. The current study showed that treatment with energy drinks increased both body weight and liver enzymes (AST, ALT and ALP) when compared to control. Power horse drink was the most effective on liver enzymes, followed by bison and code red drinks. Additionally, histological changes were observed in the hepatic tissues of treated rabbits when compared to control. These changes depend on elevated liver function enzymes. In conclusion, the consumption of energy drinks affects physiologically and histological the liver of rabbits

Keywords: : Energy drinks, rabbits, liver enzymes and histopathology.

INTRODUCTION

Energy drinks are non-alcoholic, often light soft drinks designed to provide energy by adding a number of energy-boosting ingredients. They are widely used by young people while studying, doing sports and driving long distances (Bigard, 2010).

The consumption of energy drinks containing a large amount of caffeine, taurine and carbohydrates with guarana, ginseng and B-complex vitamins (Usman, 2012 and Ballard et al. 2010) is increasing, especially among young people. It is even more alarming with the trend that is penetrating into the adult and elderly population. It is estimated that the consumption of energy drinks among adolescents and middle-aged people between 2001-2008 ranged between 24 and 56.3%. A survey of energy drink consumption among student athletes in Ghana revealed that 62.2% consumed at least one can of energy drink in a week of which 53.6% did so to replenish energy lost after training and competition (Buxton and Hagan, 2012). Other reasons for consuming energy drinks included providing energy and fluids to the body, improving performance and reducing fatigue. In a cross-sectional study of university students in Ankara, Turkey showed that the rate of energy drink consumption was higher among arts students and those who participated in sports, those who did not eat breakfast regularly, students who smoked cigarettes, and those who drank cigarettes. Alcohol compared to others (Attila and Cakir, 2011).

Recent studies reported that, caffeine is the main component of energy drinks associated with diuresis and fluid and electrolyte balance, while taurine is associated with detoxification and bile acid conjugation. Consumption of caffeinated energy drinks has shown adverse effects on hepatocytes and an increase in creatinine (Tofovic et al. 2007), aspartate transferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in rats (Akande and Banjoko, 2011). However, taurine has inconclusive effects on kidney and liver functions (Alford

and Wescott, 2001; Childs and Wit, 2008; Scholey and Kennedy, 2004)

In 2006, the Ministry of Health in the Kingdom of Saudi Arabia with delegates from the Ministry of Commerce and Industry and the Saudi Standards and Metrology Organization (SASO) studied the specialized nutrition of energy drinks and revealed some of their side effects such as irregular heartbeat, high blood pressure and poisoning especially when consumed excessively. Despite these side effects, some companies still compete to produce and market these drinks **(**Smit and Rogers, 2002). Some of the most bought brand names in Saudi Arabia are: Red Bull, Bison, Power Horse, Bugzy, Code Red and Boom Boom. In animals, taurine can produce an increase in body weight (a sign of toxicity) and dehydration. In rats and guinea pigs, high intake is associated with liver dysfunction. Small doses of taurine can enhance the toxicity of industrial pollutants such as carbon tetrachloride (Gunja and Brown, 2012).

An alarming number of side effects and even deaths have been reported, as a result of the consumption of energy drinks. Side effects reported include arrythmia, cardiac arrest, and hepatitis (Clauson et al. 2008 and Vivekanandarajah and Waked, 2011).

Other studies indicated that consumption of energy drinks affected blood chemistry and liver enzyme activities. On the other hand, Ebuehi et al. (2011) reported that there were no obvious histopathological abnormalities in the brain and liver, while Tailor reported histopathological abnormalities in the liver (Khayyat et al. 2012).Previous research has shown histological abnormalities in many areas of the brain (Al-Sudani et al. 2018). So the present work is to study the effects associated with the use of three types of energy drinks (power horse, bison, and code red) on the physiological and histological on male rabbits (Steven, 1996).

MATERIALS AND METHODS

Experimental animals:

This study was conducted on 20 adult male rabbits, weighing 1 kg, obtained from a farm in Sakaka, Aljouf, Saudi Arabia. Rabbits were housed in plastic cages, each containing 5 animals, at controlled temperature (24 ± 1 °C), 70% relative humidity and airflow conditions with constant light and dark cycles of 12 h. Rabbits were acclimatized for one week prior to the start of the experiment, fed a balanced diet and drank enough water for the duration of the experiment; After one week of settlement, all animals were weighed (Keenan et al. 2022).

Experimental design:

Several cans of three types of energy drinks: Power horse, bison and code red were used in this experiment (purchased from a local store in Sakaka, Aljouf). The animals were randomly assigned into 4 groups (5 rabbits for each group).First group: They were given distilled water and served as a control group. The second group: They were ingested orally at a dose of 1.5 ml/100 g. b. wt. of power horse daily for five weeks. The third group: They were ingested orally with 1.5 ml/100 g b. wt. of bison daily for 5 weeks. Fourth group: They were ingested orally at a dose of 1.5 ml/100 g b. wt. of code red daily for 5 weeks (Khayyat et al. 2012).

At the end of the experiment (after five weeks of treatment), animals from each group was killed by cervical dislocation, and quickly dissected. Blood samples were collected from each rabbit by cardiac puncture for enzyme determination. Livers were isolated and placed in 10% formalin for 24 h, followed by automated tissue processing to prepare slides. All slides were stained with hematoxylin and eosin dye.

Biochemical parameters (Enzyme assays):

For enzyme determination, blood samples were collected from each rabbit by cardiac puncture method and allowed to clot. Serum was rapidly separated by centrifuging the coagulated blood at 3000 rpm for 10 min in a refrigerated Beckman Model T-6 centrifuge and processed for determination in to clean and dry tubes. Sera were stored at−20°C until assessed for biochemical parameters.

The enzymatic activities of liver proteins, ALT (alanine aminotransferase), AST (aspartate aminotransferase) in serum were determined using Stephen's method (Steven, 1996), and ALP (alkaline phosphatase) using Juliet-John's method (Juliet and John, 1996).

Histological studies:

Liver samples were immediately removed and treated for light microscopy and histological examination by standard methods. Samples were fixed in 10% neutral formalin and stained with hematoxylin and eosin (Akande et al. 2010; Li et al. 2014; Kolnes et al. 2010; Egawa et al. 2011; Liu et al. 2012 and Tan et al. 2012).

Statistical analysis:

Statistical analysis performed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test using GraphPad Prism 5 (GraphPad, Software Inc., USA). The value of significant difference will considered at $p<0.05$.

RESULTS

In this study, all groups (control group and treated groups) were weighed every week as shown in table (1) and figs. (1and2).

Table 1: Average percentage of increase in body weight of control and treated groups.

Groups	1st week	2nd week	3rd week	4th week	5th week
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
C	17.17±0.06	0.056±0.05	33.26±0.91	50.6±0.46	67.49±0.78
G1	22.16±0.026^{+a}	83.86±0.25^{++a}	133.1±1.02^{++a}	200.7±0.55^{++a}	266.5±1.02^{++a}
G2	17.19±0.06^{-b}	50.60±0.45^{++a-b}	67.49±0.78^{++a-b}	87.57±0.79^{++a-b}	100.2±0.21^{++a-b}
G3	20.17±0.015^{+a-b}	33.26±0.91^{++a-b}	50.60±0.46^{++a-b}	67.49±0.78^{++a-b}	83.86±0.25^{++a-b}

C : Control group
G1: 2nd treated with Energy Drink Power Horse
G2: 3rd treated with Bison energy drink
G3: 4th treatment with energy drinks code red

The data recorded in the first week indicate that, the average weight in the treated groups (1.22 kg for power horse, 1.17 kg for bison and 1.20 for code red) did not change much compared to the control group (1.17kg). The rate of increase in body weight for control group was (%17) and (18, 17 and17%) for the three experimental groups G1, G2 and G3 respectively fig. (2)

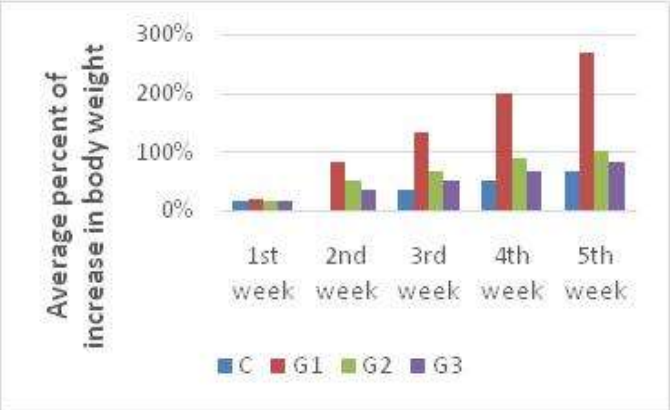

Figure 2: Average percentage of increase in body weight of control and treated groups

As for the rest of the weeks from the second week to the fifth week, it was found that the average weight in the treated groups increase a lot compared to the control group, and that the group of rabbits that was given power horse increased weight by a greater percentage than the rest of the groups, followed by bison and then the code red compared to the control group as shown in table (1) and fig. (2).

And code red on aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) on the rabbits:

Recorded data for AST, ALT, and ALP serum levels of G1 after treatment with an energy drink (power horse) was shown in table (2) and fig. (3) showed a high significant increase compared to the normal control value at $p < 0.01$.

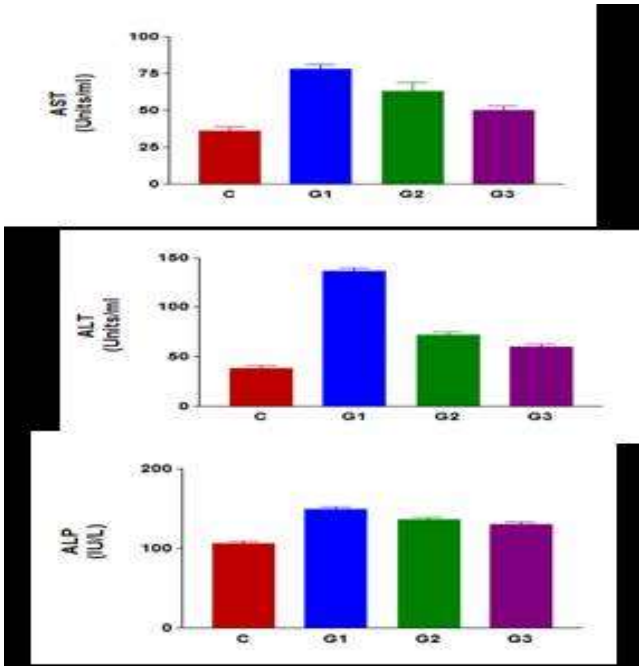

Figure 3: Effect of oral administration of power horse, bison and code red on liver function [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)] on the rabbits.

While the results in group G2 were treated with bison as shown in table (2) and fig. (3), there was a high significant increase compared to group C at $p < 0.01$ for AST, ALT and ALP, as well as a significant decrease in AST, ALT and ALP compared with group G1 at $p < 0.05$.

Table 2: Effect of oral administration of power horse, bison and code red on liver function [aspartate aminotransferase (AST), alanineaminotransferase (ALT) and alkaline phosphatase (ALP)] in the rabbits

Group	ALP (IU/L)	ALT (Units/ml)	AST (Units/ml)
	Mean ±S.D.	Mean ±S.D.	Mean ±S.D.
C	106 ± 5	38 ± 5	36 ± 5
G1	149^{++a}± 5	136 $^{++a}$ ± 5	78 $^{++a}$ ± 5
G2	136^{++a-b}± 5	72 $^{++a--b}$ ± 5	62.67 $^{++a-b}$± 5
G3	130^{++a-b}± 5	60 $^{++a- -b}$± 5	50 $^{+a--b}$ ± 5

++a: highly significant increased compared with C group at p<0.01.
+a: significant increased compared with C group at p<0.05.
- -b: highly significant decreased compared with G group at p<0.01.
-b: significantly decreased compared with G group at

p<0.05.

As shown in Table No. (2) Fig. (3), it is evident that the liver function (AST) in the code red-treated G3 group was significantly increased compared with group C at P<0.05 and a highly significant decreased compared with the G1 group at P<0.01. While the results of ALT showed a high significant increase compared with the C group at p < 0.01, and a high significant decrease compared with the G1 group at p < 0.01. Also, the level of alkaline phosphatase was a highly significant increased compared to group C at p < 0.01 and decreased significantly compared to group G1 at p < 0.05.

Histological studies:

Histological section in the liver of control rabbits which shows a normal liver design without any obvious lesion was seen in Plate 1 (1).Treated rabbit livers in all groups showed different stages of histopathological changes. In group (G1), power horse consumed energy drink for 35 days, sections showed that hepatocyte cords were irregular with hydropic degeneration between the sinuses. Fat degeneration and sometimes scattered areas of necrosis appear where the hepatic sutures are no longer aligned. Necrosis is usually accompanied by the appearance of lymphocyte infiltration throughout the hepatic tissue and portal tract. In addition to the above lesions, engorged blood vessel is also seen in Plate 1 (2).

In Plate 1 (3),histological liver sections of group (G2), consuming bison energy drink, have lymphocytic infiltration (L) and karyorrhexis nuclei (karyorrhexis shows fragmentation of the chromatin network into several deeply stained fragments located adjacent to the nuclear membrane which soon disappear leaving the chromatin fragments well distributed irregular in the cytoplasm).

Histopathological changes in the liver section of the (G3) group, consuming code red energy drink were less than other treated groups such as dilated and congested blood vessel as shown in Plate 1 (4).

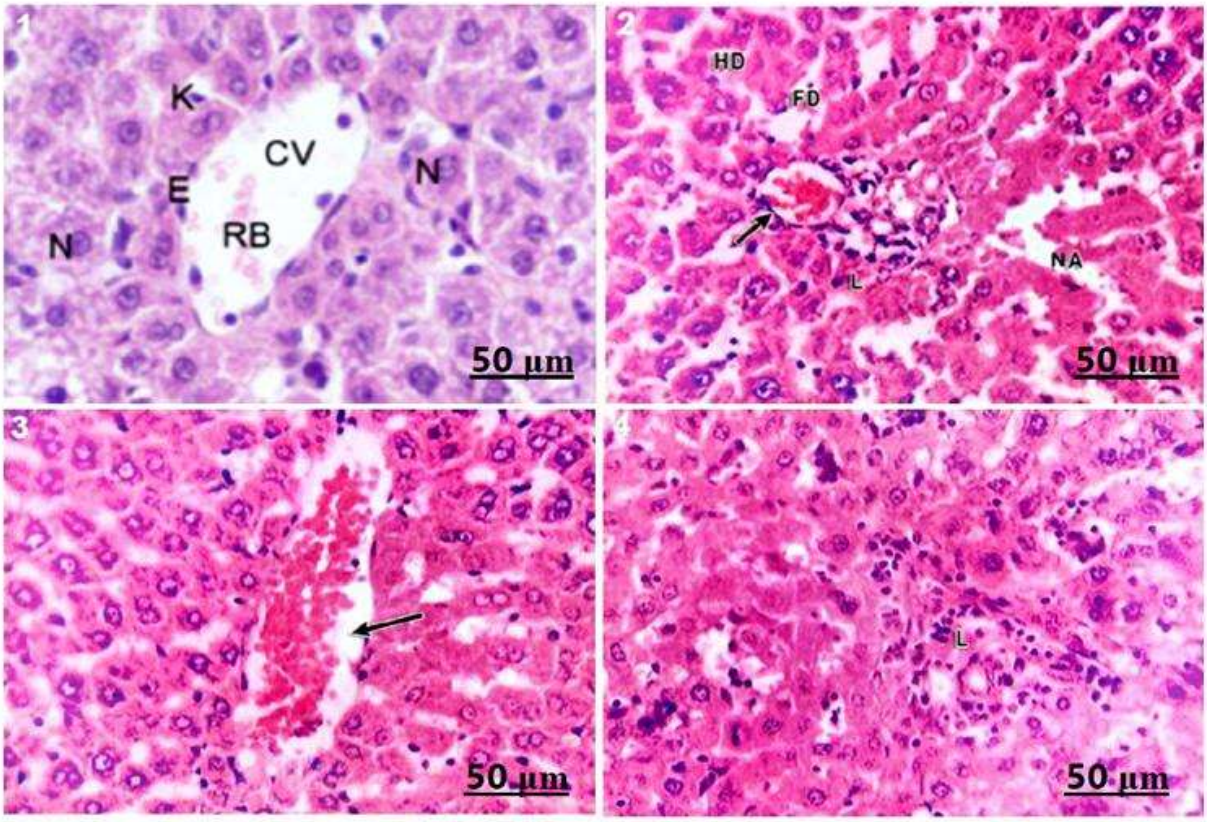

Plate 1: Showing (1):Light micrograph of liver section from control rabbit to show the normal histological structure ofhepatocytes with central spherical nucleus (N), Central vein (CV) lined with endothelial cells (E), Kupffer cells (K) and contained red blood cells (RB). S: HX andE. X 200, (2): Light micrograph of liver section from rabbit given power horse drink for 5 weeks showing hydropic degeneration (HD), fatty degeneration (FD), congested blood vessel (→), lymphocytic infiltration (L) and necrotic area (NA). S: HX andE. X 200, (3): Light micrograph of liver section from rabbit given bison for 5 weeks showing lymphocytic infiltration (L)and karyorrhexis nuclei (←). (S: HX andE. X 200), and ((4): Light micrograph of liver section from rabbit given code red for 5 weeks showing dilated and congested blood vessel (arrow). HandE. x400.

DISCUSSION

The current study showed some effects on rabbits as a result of giving them different energy drinks, including:

Effect of energy drinks on body weight:

In the present work, the first noticeable effect of energy drinks on rabbits was an increase in body weight gain in the experimental groups compared to the control group, and the largest weight in the energy drink-treated group was power horse, followed by the bison, then the code red. These results are in accordance with other studies reported by (Li et al. 2014), where they stated that sugar added to energy drinks may be responsible for obesity. Additionally, animal studies have shown that caffeine increases insulin resistance and disrupts glucose metabolism (Kolnes et al. 2010; Egawa et al. 2011 and Liu et al. 2012). Also, (Tan et al. 2012) statedthat an energy drink alone or in combination with alcohol has variable effects on kidney function and liver enzymeswith a combination of energy drink and alcohol having more effects than energy drinks only, excepting for body weight where energy drink only has higher effects.

Biochemical Parameters (serum levels of AST, ALT and ALP):

On the other hand, this study showed that oral administration of energy drinks to rabbits for 5 weeks significantly increased serum levels of AST, ALT, and ALP. Increases in the levels of hepatic enzymes in the blood serve as reliable indicators of liver damage due to toxic agents. Similar increases in serum AST, ALT, and ALP were reported for rats exposed to energy drinks containing caffeine(Ballard et al. 2010).It has been shown that rats that consumed energy drinks alone or in combination with alcohol showed higher serum total bilirubin, ALT, ALP, and AST than untreated controls (Bukhari et al. 2012). Another study revealed that, elevated levels of the liver function enzymes GOT, GPT, and ALP in rats that consumed the energy drink, which is in agreement with several studies (Ugwuja, 2014andHuang et al. 2014).Energy drinks typically contain 80-141 mg of caffeine per 8-ounce serving, which is equivalent to five ounces of coffee or two 12-ounce cans of caffeinated soda (Alkhedaide et al. 2016). This higher concentration of caffeine caused GOT and GPT levels to rise in rats (Pronsky, 1997),while other studies reported that caffeine caused GOT to be lowered(Cheul et al. 1997 and Ruhl andEverhart, 2005).

According to Cadden et al. (2007)energy drink consumption was associated with higher total protein, triglycerides, HDL and LDL but lower ALT, AST, creatinine, uric acid and albumin. This is in contrast to (Ebuehi et al. 2011)who reported significantly higher liver enzymes in animals treated with energy drinks. Also, (Ugwuja, 2014)reported that, energy drink consumption alone or in combination with alcohol is associated with significant changes in some biochemical parameters such as total white blood cell count, plasma potassium, calcium, renal function, liver enzymes and plasma triglycerides. Liver function enzymes, ALT, AST and ALP were elevated in the serum of rats after treatment with each of the energy drinks. This is in agreement with results of (Akande and Banjoko, 2011)who reported that there was an increase in serum AST, ALT and ALP in power horse-treated rats. It was also found in (Ebuehi et al. 2011) that, power horse and red bull significantly influence liver enzyme activities in rabbits.

Histopathological parameters:

The current study also showed that the ultrastructural findings and their correlation with histopathological findings demonstrated that oral consumption of energy drinks caused changes in liver cells.

The pattern of differences observed in the liver function parameters of rats exposed to different doses of energy drinks was in agreement with the lesions on the microscopic images of these tissues. The observed lesions are likely the result of the harmful effects of energy drinks. It can reasonably be said that the lesions were caused by tissue damage from energy drinks that cause oxidative stress. These results are consistent with a previous report of evidence of hepatotoxicity and changes in liver ultrastructure in rats treated with different types of energy drinks (Khayyat et al. 2012). In another study, lesions in liver and kidney tissue were attributed to a possible interaction of taurine with some other active energy drink ingredients such as caffeine (Bergerand Alford, 2009).Additionally, Khayyat and his colleagues found that rats treated with energy drinks had hepatic cytoplasmic vacuoles due to the presence of lipid droplets attributable to degenerative changes within hepatocytes (Khayyat et al.2012).

Several researchers agree on the adverse effects of energy drinks as obtained in this study (Bukhari et al. 2012). However, others have reported that power horse and red bull significantly affected liver enzyme activities but had no significant effect on liver histopathology (Ebuehi et al. 2011). Some investigators have reported irregular outlines of hepatocytes, pyknosis in the nuclei of hepatocytes, and several mitotic forms (Akande and Banjoko, 2011). These changes may be attributed to the toxic effects of caffeine, and the harmful effects of preservatives added to energy drinks, such as sodium benzoate (Mubarak, 2012).

Khayyat et al. (2012) and Mubarak (2012) studies showed that the cytoplasm of hepatocytes of rats, which consumed energy drinks, appeared vacuolized with the presence of lipid droplets. These can be attributed to degenerative changes within hepatocytes.

On the other hand, several works have studied the structural alterations of hepatocytes of animals consuming

energy drinks and have reported the dilatation and fragmentation of rough endoplasmic reticulum cisternae (Kroder et al. 1996),which may lead to hepatocyte damage (Sato et al. 1999; Kumar et al. 2005andTasci et al. 2008).In addition, (Balaban et al. 2005) noted a deterioration in mitochondrial function due to a disturbance in mitochondrial structure.Mubarak's case study showed irregular outlines, pyknosis and several mitotic forms in the nuclei of hepatocytes in(Mubarak, 2012), he attributed these changes to preservatives added to energy drinks such as sodium benzoate, and to the toxic action of caffeine These changes could lead to the hepatocellular necrosis indicated in this current study.

CONCLUSION

It can be concluded that the consumption of energy drinks has a histological and physiological effect on rabbit liver. Care must be taken while taking it. There is an urgent need for public health education to correct the wrong impression already formed by unsuspecting consumers, especially young people.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

FMZ and MSA designed and performleed the experiments and also wrote the manuscript. AA, FMZ, HTH, and HIE performed animal treatments, flow cytometry experiments, tissue collection, and data analysis. FMZ and MSA reviewed the manuscript. All authors read and approved the final version.

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