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Biological Effect of High Energy Drink on Normal and Hyperglycemic Rats

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Abstract: Several studies suggest that there was relationships between energy drink consumption and problem behaviors among adolescents and adults as it increase lipolysis glycogenolysis and catecholamine secretion. This study aimed to find out the potential effects of high energy drinks recommended intake and toxic dose on normal and hyperglycemic rats. Thirty-six (36) male adult Sprague-Dawley rats weighting 145±5.3 g each were used in this investigation. Non-diabetic rats [control-ve 6 rats feed on basal diet only and 12 Normal Rats (NR) divided into two groups consumed basal diet with 1 and 2 ml of High Energy Drink (HED) by gastric tube], while Diabetic Rats (DR) control+ve 6 rats received basal diet only and 12 rats divided into two groups consumed basal diet with 1 and 2 ml of HED after injected with alloxan for inducing diabetes mellitus. Body Weight Gain (BWG) and food intake were recorded weekly for 6 weeks. Blood samples were collected after 12 hours fasting at the end of experiment. Liver was removed and weighted. Blood serum was prepared for measurements of glucose, triglyceride, cholesterol, HDL-c, LDL-c, VLDL-c, AST, ALT and ALP. The BWG of NR groups received 2 ml only and DR groups received 1 and 2 ml of HED by oral injection recorded significant decrease ($p<0.001$) as compared to the control negative group. Blood glucose level was significantly higher ($p<0.001$) for DR fed on 1 and 2 ml compared with control (-). Serum AST, ALT and ALP were significantly higher ($p<0.01$ and $p<0.001$ resp.) for NR received the two doses of HED compared with normal rats control (-). As for cholesterol, triglycerides and LDLc levels were significantly higher ($p<0.01$) in the hyperglycemic rats group fed on 2 ml of HED compared with control (-). Also LDLc/HDLc ratio increased gradually when the level of HED increased. Oral injection by HED cause histopathological changes in the liver for NR and DR like atrophy and cell damage also changes in the chemical and morphological structure.

Key words: High energy drink, diabetic rats, glucose, serum lipids, liver function, histopathological

INTRODUCTION

Energy drinks have become more popular since the late nineties. The manufactures claim that these drinks improve physical endurance, reaction speed and concentration. The main ingredients of energy drinks are caffeine, sugar, taurine and glucuronolactone (Van den Eynde *et al*., 2008). The 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 (Beck *et al*., 2006). Recent literature suggests that both caffeine and taurine can induce diuresis and natriuresis in rat and man (Riesenhuber *et al*., 2006). According to the manufacturers, the stimulating effects of these drinks are due to interaction between the various ingredients (Van den Eynde *et al*., 2008). Another study by Forbes *et al*. (2007) found that *Red Bull* energy drink significantly increased upper body muscle endurance but had no effect on anaerobic peak or average power during repeated Wingate cycling tests in young healthy adults. Smit *et al*. (2006) illustrate the restorative combination of caffeine and CHO in the drink and emphasizes the need to implement the appropriate placebo (s) in any study design employing familiar foods or drinks. Scholey and Kennedy (2004) reported that 100 ml that contain carbohydrate 12g, calories 51kcal and caffeine 24g can improve aspects of cognitive performance and in the case of caffeine, mood. However several studies suggest that energy drinks may serve as a gateway to other forms of drug dependence. Regulatory implications concerning labeling and advertising and the clinical implications for children and adolescents are discussed (Reissig *et al*., 2009). Consumption of Sugar-Sweetened Beverages (SSBs) has been linked to the obesity epidemic (Malik *et al*., 2006), which currently affects one-third of US adults (Ogden *et al*., 2006; Wang and Beydoun, 2007) and type 2 diabetes (Schulze *et al*., 2004). In Arabic world this drink became more acceptable for young adult at pre-puberty stage and college students. There are few studies investigating the effects of the two substances in combination. Therefore, this investigation was aimed to study the biological effect of energy drinks on normal and alloxan diabetic rats.

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MATERIALS AND METHODS

The studied sample is high energy drink, (we do not mention the name for legal protection) the nutritional facts for serving size (100 ml) that contain carbohydrate 12 g, calories 51 kcal and caffeine 24 g. Food item has been bought from local market at Makkah Governorate.

Alloxan: Pure chemical fine (BDH, obtained from Sigma) was used for inducing diabetes in this study. Untreated rats are referred to as the control negative (control-group), while alloxan treated rats are the control positive (control + group); these are groups 1 and 2 respectively.

Animals: A total of 36 adult male albino rats (*Sprague Dawley strain*) an average weight (145±5.3 g) were used in the investigation. The animals were obtained from animal house of Biochemistry department, Faculty of medicine Umm Al Qura University. Each rat was housed in special cage under controlled condition. Every day the animals were observed for the external appearance, shape, distribution of hair and physical activity. All rats were fed for 3 days on the control diet before the beginning of the experiment. The rats were weighed after 3 days (each separately) then once a week for 6 weeks. The diet was presented to rats in special covered cups to avoid food loss. All rats were provided with water by glass tubes through wire cage.

Standard diet: The basal diet consists of 20% casein, 5% corn oil, 5% cellulose, 1% vitamin mixture, 3.5% salt mixture, 0.2% choline chloride and the remainder (65%) is corn starch (Reeves *et al*., 1993). Vitamin composition of diets prepared according to AOAC (1975).

Biological studies

Preparation of diabetic rats: Diabetes was induced in normal healthy male albino rats via intraperitoneal injection of alloxan (150 mg/kg body weight) according to the method described by Desai and Bhide (1985). Six hours after the injection of alloxan, fasting blood samples were obtained by retro-orbital method to estimate fasting serum glucose. Rats having fasting serum glucose more than 160 mg/dl were considered diabetics NDDG (1994).

Field protocol: Rats were divided into two groups (normal and diabetic) as follow:

Normal rats NR: Group 1 rats fed on basal diet (control negative) and Group 2 and 3 NR fed on basal diet + 1 and 2 ml of HED respectively.

Diabetic rats DR: Group 4 Diabetic rats fed on basal diet (control positive) and Group 5 and 6 DR fed on basal diet + 1 and 2 ml of HED respectively.

Blood sampling: At the end of the experiment, rats were fasted over night and anesthetized with chloroform. Blood samples were collected in clean dry centrifuge tubes from portal vein. Blood was centrifuged for 10 minutes at 3000 rpm sample were kept at-18°C till analysis. Internal organs were removed, washed with 5% saline and dried with filter paper, then weighted according to Chapman *et al*. (1959).

Changes in body weight and dietary intake: All rats were weighted once weekly. At the end of the experiment, biological evaluation of the different diets was carried out by determination of body weight gain% (BWG%) and Food Efficiency Ratio (FER) according to Chapman *et al*. (1959).

Biochemical analysis: Enzymatic colorimetric method used to determine blood glucose according to Trinder (1969). Colorimetric method was used for the determination of total cholesterol according to Allain (1974). Determination of HDLc was carried out according to the method of Friedewald (1972) and Gordon and Amer (1977). Enzymatic colorimetric method used to determine triglycerides according to Young and Pestaner (1975). The determinations of VLDLc and LDLc were carried out according to the method of Lee and Nieman (1996) to find the concentration of very low density lipoprotein by these equation (VLDLc = triglycerides/5) and LDLc = total cholesterol-(HDLc + VLDLc). Colorimetric method used to determine AST and ALT according to Reitman and Frankel (1957); while determination of alkaline phosphatase ALP activity according to Haussament (1977).

Histopathological study: Liver specimens only were collected from rats of all experimental groups at the end of the experimental period, fixed in 10% neutral buffered formalin (pH = 7.0), dehydrated in ethyl alcohol, then cleared in xylol and embedded in paraffin; 4-6 microns thickness sections prepared and stained with heamtoxylin and eosin for examining the liver using light microscope at various magnification (Carleton, 1976).

Statistical analysis: Statistical analysis has been achieved by using statistical package for social science program (SPSS, 2008).

RESULTS

The effect of High Energy Drink (HED) with two levels on Food Intake (FI), BWG% and FER for normal and hyperglycemic rats as shown in Table 1. FI (g/day) did not differ significantly between the control normal rats NR group fed on diet containing 1 ml of HED and the negative control group (fed on basal diet). The results indicated that, significant differences in FI were observed

Table 1: Mean±SD values of Food Intake (FI), Body Weight Gain (BWG) and Food Efficiency Ratio (FER) for normal and diabetic rats with/without high energy drink

Groups variables	NR (C-)	NRCHED 1 ml	NRCHED 2 ml	DR (C+)	DRCHED 1 ml	DRCHED 2 ml
FI (g)	13.9±0.8	14.1±0.3	15.1±0.8*	11.7±1.4*	11.6±0.8**	10.1±0.2***
BWG (%)	9.1±1.8	9.4±2	0.1±0.04**	0.4±0.1***	0.1±0.31***	2.6±0.32***
FER	0.012±0.02	0.019±0.006	0.0009±0.1	0.001±0.0002	0.004±0.002	0.008±0.002

NR: Normal Rats; DR: Diabetic Rats; NRCHED: Normal Rats Consumed High Energy Drink; DRCHED: Diabetic Rats Consumed High Energy Drink. *Differences are significant at 5% (p<0.05). **Differences are significant at 1% (p<0.01). ***Differences are significant at 0.1% (p<0.001)

Table 2: Mean±SD values of liver for normal and diabetic rats with/without high energy drink

Groups variables	NR (C-)	NRCHED 1 ml	NRCHED 2 ml	DR (C+)	DRCHED 1 ml	DRCHED 2 ml
Liver (g)	4.9±0.4	5.8±04*	7.3±0.7**	7.1±1	7.5±0.4*	7.6±0.3**
Liver/body	0.033±0.003	0.038±0.003*	0.046±.004**	0.041±0.007	0.04±.002	0.05±0.001*

NR: Normal Rats; DR: Diabetic Rats; NRCHED: Normal Rats Consumed High Energy Drink; DRCHED: Diabetic Rats Consumed High Energy Drink. *Differences are significant at 5% (p<0.05). **Differences are significant at 1% (p<0.01). ***Differences are significant at 0.1% (p<0.001)

Table 3: Mean±SD values of AST, ALT and ALP (U/L) for normal and diabetic rats with/without high energy drink

Groups variables	NR (C-)	NRCHED 1 ml	NRCHED 2 ml	DR (C+)	DRCHED 1 ml	DRCHED 2 ml
AST	41.1±2.2	115±5.4**	131±5.3***	117.5±3.1**	139±5.9***	166±3.2***
ALT	42.4±2.2	88.1±6**	85.6±18.7**	76.3±15.1**	92.6±1.2**	102.3±6.3***
ALP	115.5±3.1	178±9.8**	185±5.4***	226±11.6***	238.5±1.5***	349.2±34***

NR: Normal Rats; DR: Diabetic Rats; NRCHED: Normal Rats Consumed High Energy Drink; DRCHED: Diabetic Rats Consumed High Energy Drink. *Differences are significant at 5% (p<0.05). **Differences are significant at 1% (p<0.01). ***Differences are significant at 0.1% (p<0.001)

of all DR groups fed on HED, as compared to the negative control group respectively. Meanwhile, increase of HED dose lead to significant decrease in FI for DR groups compared to C-ve and C+ve. The mean values of BWG% decreased significantly (p<0.001) in the positive DR group compared to the negative control group (0.4±0.1 vs. 9.1±1.8, respectively). Also BWG% of NR groups fed on diet containing 1 and 2 ml of HED recorded significant decrease p<0.001, as compared to the negative group (0.1±0.31, 2.6±0.32 and 9.1±1.8% respectively). While, the results for NR group fed on 1 ml of HED indicated that, non-significant increased when compared with the negative control group (9.4±2 vs. 9.1±1.8% respectively), while highly significantly decreased in p<0.01 was observed for NR group fed on 2 ml of HED (0.1±0.04%).

The results in Table 2 showed that, the mean values of liver weight (g) and Liver/body changes for NR and DR groups with or without HED. Liver weight of alloxan DR groups were higher than in NR groups. This is also was clearly that feeding on basal diet supplemented with 2 ml of HED caused a significant increase in average weight of liver compared with negative control group (p<0.01).

Figure 1 showed the mean values of fasting serum glucose (mg/dl) for normal and diabetic rats consumed HED. Blood glucose was significantly increased (p<0.001) in alloxan induced diabetic rats compared with negative control (192.5±4.2 and 88.8±3.1 mg/dl resp.). In the same time, blood glucose levels were significantly higher (p<0.001) for DR received 1 and 2 ml compared

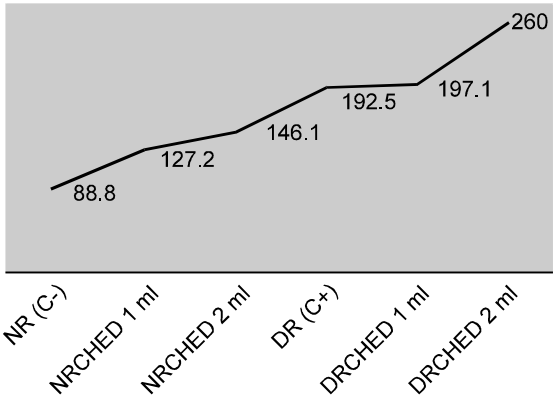

Fig. 1: Fasting serum glucose (mg/dl) for normal and diabetic rats consumed high energy drink

with negative control (197.1±1.2, 260±36.3 and 88.8±3.1 mg/dl resp.), Also it was significantly higher (p<0.01) for normal rats fed on the same levels of HED (127.2±9.5 and 146.1±4.9 mg/dl resp.).

Data in Table 3 showed the mean values of fasting serum glucose (mg/dl), AST, ALT and ALP (U/L) for normal and diabetic rats consumed HED. The obtained data revealed that serum AST, ALT and ALP were increased in positive diabetic rats compared with negative control rats. Serum AST, ALT and ALP were significantly higher (p<0.01 and p<0.001 resp.) for normal rats received two levels 1 and 2 ml HED compared with negative control rats. The same trend was observed also for diabetic groups.

Table 4: Mean±SD values of fasting serum lipids profile (mg/dl) for normal and diabetic rats with/without high energy drink

Groups variables	NR (C-)	NRCHED 1 ml	NRCHED 2 ml	DR (C+)	DRCHED 1 ml	DRCHED 2 ml
Cholesterol	94.5±4.5	101.5±5.6*	102.1±3.9*	107.5±5.5*	109.4±5.1**	122.9±8.5**
Triglycerides	85.7±3.4	91.3±1.9	92.4±1.5*	92.3±2.1	97±9.5*	117.2±7.9**
HDLc	36.5±4.5	32.2±2.1	31±5.4	32.1±6.8	30.8±5.4*	31.2±8.3
VLDLc	17.1±0.7	18.3±4.6	18.5±4.1*	18.5±4.2*	19.4±4.2*	23.4±3.2*
LDLc	40.9±4.9	51±0.4	52.6±4.3*	56.9±5.4*	59.2±6.7*	68.3±3.5**

NR: Normal Rats; DR: Diabetic Rats; NRCHED: Normal Rats Consumed High Energy Drink; DRCHED: Diabetic Rats Consumed High Energy Drink. *Differences are significant at 5% ($p<0.05$). **Differences are significant at 1% ($p<0.01$). ***Differences are significant at 0.1% ($p<0.001$)

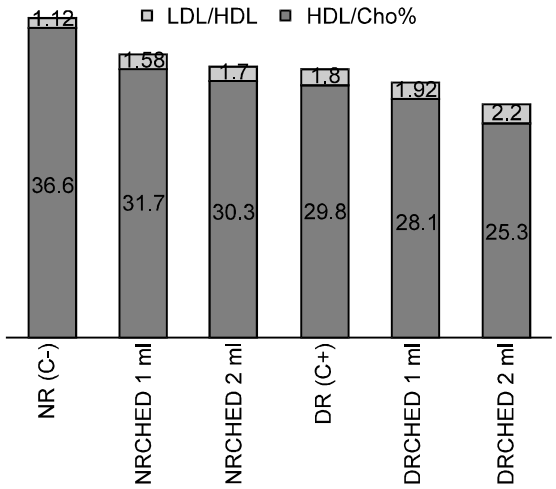

Fig. 2: Atherogenic indices for normal and diabetic rats fed on high energy drink

Table 4 showed that mean values of serum lipids profile (mg/dl) for normal and diabetic rats consumed HED. Results revealed that cholesterol, triglycerides and LDLc were significantly higher ($p<0.01$) in the hyperglycemic rats group fed on 2 ml of HED compared with negative control. The same trend was observed for NR group fed on 2 ml of HED ($p<0.05$). Also serum lipids profile for positive control were significantly higher ($p<0.05$) compared with negative control group, meanwhile HDLc was non-significantly lower for positive control compared with negative control group (32.1±6.8 and 36.5±4.5 mg/dl resp.).

Data presented in Fig. 2 showed total LDLc/HDLc and HDLc/total cholesterol ratios as atherogenic indices for normal and hyperglycemic rats fed on HED. The lower of HDLc/total cholesterol ratio reflect the increased risk of Coronary Heart Disease (CHD), meanwhile amplified of LDLc/ HDLc ratio indicated the enlarged risk of CHD. It is worth to notice that NR groups which received 1 and 2 ml of HED showed increased of LDLc/HDLc ratio compared with the negative control (1.58, 1.7 and 1.12 resp.), also the same results was observed in hyperglycemic groups which received the same levels of HED (1.58, 1.92 and 2. 2 resp.). It is obvious to notice that LDLc/HDLc ratio increased gradually when the level of HED increased as shown in Fig. 2. The same trend was observed for HDLc/total cholesterol ratios, the same levels showed the reduction of HDLc/cholesterol ratio compared with the negative control, normal rats groups were (31.7, 30.3 and 36.6 resp.) and hyperglycemic groups (28.1, 25.3 and 36.6 resp.).

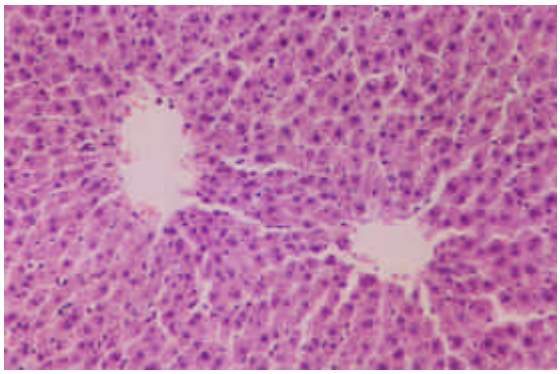

Fig. 3: Liver of control untreated rat showing the normal histology of hepatic lobule (H and E x 200)

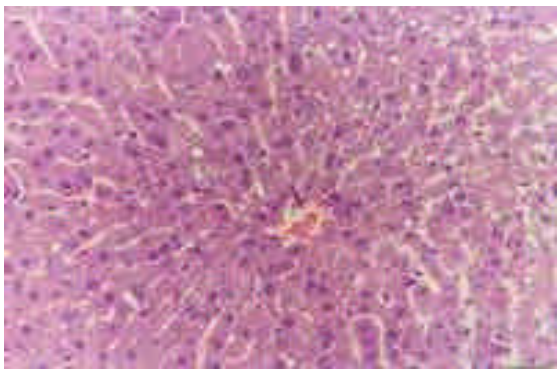

Fig. 4: Liver of diabetic rat showing vacuolar degeneration of some hepatocytes and pyknotic cells (H and E x 200)

Histopathological results: Examined liver of control, untreated rat revealed the normal histology of hepatic lobule, which consists of central vein and around it concentrically arranged highly specialized cells (hepatocytes) (Fig. 3). Concerning liver of diabetic rat, it showed vacuolar degeneration of some hepatocytes (empty foci devoid of liver tissue) as well as focal hepatic hemorrhage, kuppffer cell activation, pyknotic cells and dilatation of hepatic sinusoids (Fig. 4, 5 and 6).

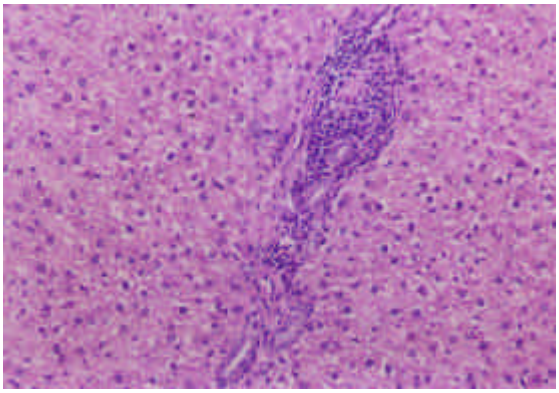

Fig. 5: Liver of diabetic rat showing kuppffer cell activation (H and E x 200)

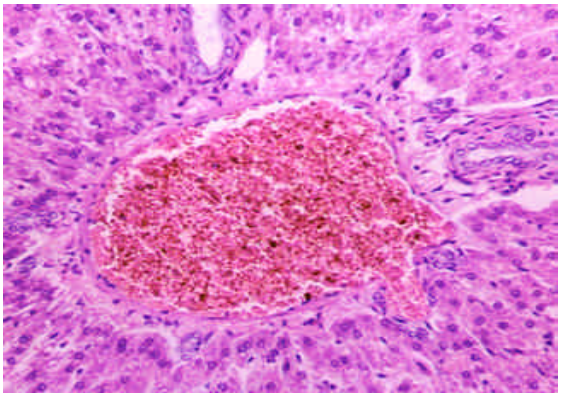

Fig. 6: Liver of diabetic rat showing focal hepatic hemorrhage and dilatation of hepatic sinusoids (H and E x 200)

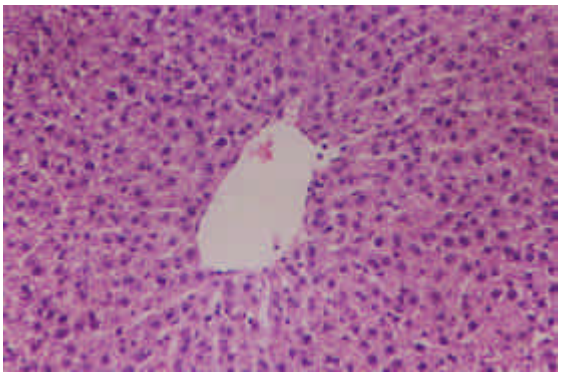

Fig. 7: Microscopically examination of liver for NR that consumed 1 ml of HED revealed no changes except small vacuoles in the cytoplasm of some hepatocytes (H and E x 200)

Microscopically examination of liver for NR that consumed 1 ml of HED revealed no changes except small vacuoles in the cytoplasm of some hepatocytes (Fig. 7). However, liver for NR fed on 2 ml of HED showed hepatocellular vacuolations (hydropic degeneration), many hepatocytes appeared non nucleated and focal leucocytic cells infiltration (Fig. 8). Examined liver for DR consumed 1 ml of HED revealed dilatation and congestion of central veins and hepatic sinusoids as well as intravascular leucocytes, pyknotic cells and sporadic necrosis of hepatocytes (Fig. 9 and 10). Diabetic rats consumed 2 ml of HED revealed signs of liver hemorrhage and progressive hepatocyte deformities with massive necrosis, dilatation and congestion of hepatoportal blood vessels, hyper dilatation of hepatic sinusoids portal edema and hemorrhage associated with hyper deposition of collagen fibers in the portal triad and disappearance of normal organization of liver tissues specially the lining cells which became scattered in the nearest parenchyma (zigzag shape) (Fig. 11 and 12).

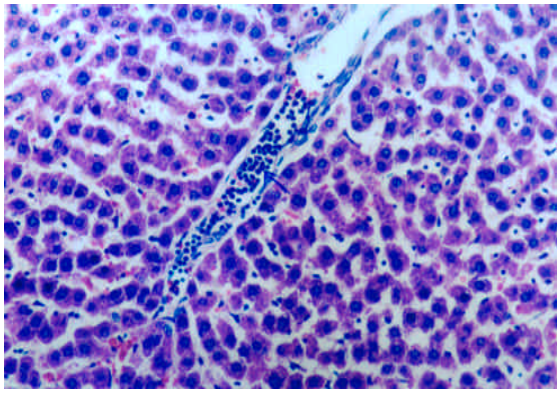

Fig. 8: Liver for NR fed on 2 ml of HED showed hepatocellular vacuolations (hydropic degeneration), many hepatocytes appeared non nucleated and focal leucocytic cells infiltration (H and E x 400)

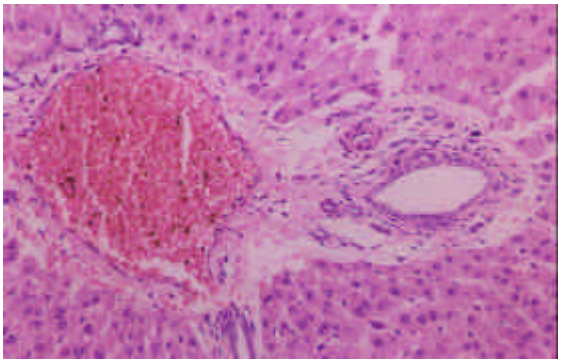

Fig. 9: Liver of DR fed on 1 ml of HED showed dilatation and congestion of central veins and hepatic sinusoids as well as intravascular leucocytes, pyknotic cells and sporadic necrosis of hepatocytes (H and E x 200)

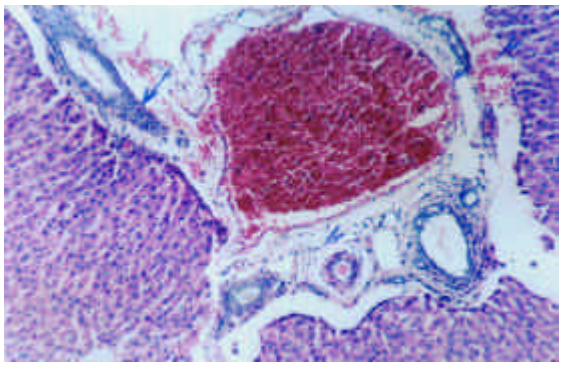

Fig. 10: Liver of DR fed on 1 ml of HED showed dilatation and congestion of central veins and hepatic sinusoids as well as intravascular leucocytes, pyknotic cells and sporadic necrosis of hepatocytes (H and E x 200)

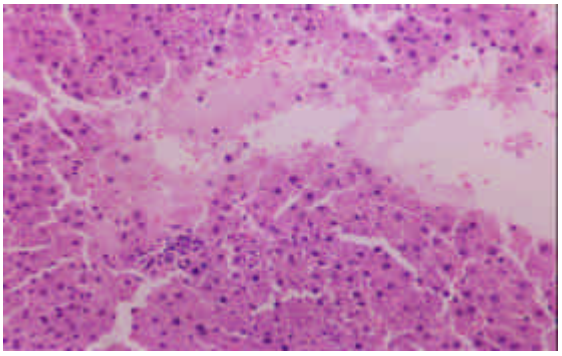

Fig. 11: Liver of DR fed on 2 ml of HED revealed marked dilatation and congestion of hepatoportal blood vessels, hyper dilatation of hepatic sinusoids portal edema (H and E x 200)

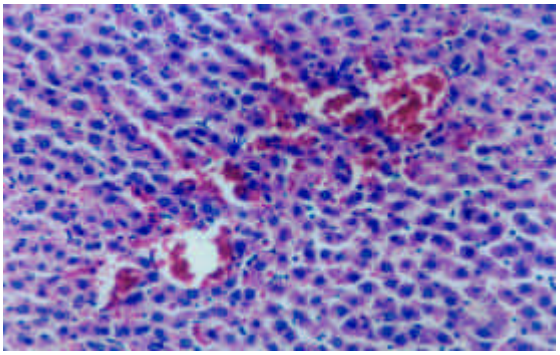

Fig. 12: Liver of DR fed on 2 ml of HED showed hemorrhage associated with hyper deposition of collagen fibers in the portal triad and disappearance of normal organization of liver tissues specially the lining cells which became scattered in the nearest parenchyma (zigzag shape) (H and E x 400)

DISCUSSION

Our results revealed that BWG% decreased significantly ($p<0.001$) in the positive DR group compared to the negative control group. Also BWG % of NR groups fed on diet containing 1 and 2 ml of HED recorded significant decrease $p<0.001$, as compared to the negative group. While, the results for NR group fed on 1 ml of HED indicated that, non-significant increased when compared with the negative control group, while highly significantly decreased in $p<0.01$ was observed for NR group fed on 2 ml of HED. In this respect Header (2006) reported that alloxan injection caused a significant decrease in average BWG in rats. Also Mohamed (2000) found that all rats, except negative control, lost weight after injection by alloxan. Concerning of FER the data revealed that no significant differences in the FER for negative group compared with other NR and DR groups. Libuda and Kersting (2009) reported that the high sugar content of regular soft drinks could have an influence on energy balance and body weight especially in childhood and adolescence.

In another ward the HED are designed to enhance our activities hence gain weight diminished, some side effects of caffeine are anxiety, disturbance of GIT this can explain the less food intake in the diabetic group Boyle and Castillo (2006). Energy drinks typically contain 80 to 141 mg of caffeine per 8 ounces, the equivalent of five ounces of coffee or two 12-ounce cans of caffeinated soft drink such as Mountain Dew, Coca Cola, Pepsi Cola (Pronsky, 1997). Caffeine toxicity is defined by specific symptoms that emerge as a direct result of caffeine consumption. Common features of caffeine intoxication include nervousness, anxiety, restlessness, insomnia, gastrointestinal upset, tremors, tachycardia, psychomotor agitation (Kerrigan and Lindsey, 2005).

These results are in accordance with those of Mohamed Hala (2004) she noticed that liver weight of alloxan DR groups were higher than in NR groups. This is also was clearly that feeding on basal diet supplemented with 2 ml of HED caused a significant increase in average weight of liver and spleen compared with negative control group ($p<0.01$ and $p<0.05$ resp.) The increase in liver and spleen weight appears to due to the diabetogenic effect of alloxan which resulted in insulin deficiency. On the other hand, all the organs weight for the diabetic groups were higher than the negative group. Meanwhile the changes in liver weight could be referred to the basal diet supplemented with 1 and 2 ml of HED compared with the negative control also normal rats also supplemented with 1 and 2 ml of HED showed large changes in liver weight compared with the negative control.

As for Liver weight of alloxan DR groups were higher than in NR groups. These results are in accordance with those of Mohamed Hala (2004). This is also was clearly that feeding on basal diet supplemented with 2 ml of

HED caused a significant increase in average weight of liver compared with negative control group ($p<0.01$). The increase in liver weight appears to due to the diabetogenic effect of alloxan which resulted in insulin deficiency. Meanwhile the changes in liver weight could be referred to the basal diet supplemented with 1 and 2 ml of HED compared with the negative control also normal rats also supplemented with 1 and 2 ml of HED showed large changes in liver weight compared with the negative control.

Our results revealed that blood glucose was significantly increased ($p<0.001$) in alloxan induced diabetic rats compared with negative control. In the same time, blood glucose levels were significantly higher ($p<0.001$) for DR received 1 and 2 ml compared with negative control. Also it was significantly higher ($p<0.01$) for NR fed on the same levels of HED. These results due to when a high level of sugar is in the blood stream the body cannot get the water into the cells that it needs because the water is In this respect Bleich *et al*. (2009) reported that consumption of Sugar-Sweetened Beverages (SSBs) has been linked to obesity and type 2 diabetes. Also Bleich *et al*. (2008) found that SSB comprises a considerable source of total daily intake and is the largest source of beverage calories. SSB consumption is highest among subgroups also at greatest risk of obesity and type 2 diabetes.

The obtained data revealed that serum AST, ALT and ALP were increased in positive diabetic rats compared with negative control rats. These results supported by Shoukry and Rhab (2006). Serum AST, ALT and ALP were significantly higher ($p<0.01$ and $p<0.001$ resp.) for normal rats received two levels 1 and 2 ml compared with negative control rats. The same trend was observed also for diabetic groups. In another study by Lee *et al*. (2005) which mentioned that the acute caffeine consumption reduces insulin sensitivity. Also Bichler *et al*. (2006) stated that HED causes increase mean arterial blood pressure. Regarding central nervous system, cardiovascular, gastrointestinal and renal dysfunction have been associated with chronic caffeine ingestion (Carrillo and Benitez, 2000).

Results revealed that cholesterol, triglycerides and LDLc were significantly higher ($p<0.01$) in the hyperglycemic rats group fed on 2 ml of HED compared with negative control. The same trend was observed for NR group fed on 2 ml of HED ($p<0.05$). Also serum lipids profile for positive control were significantly higher ($p<0.05$) compared with negative control group, meanwhile HDLc was non-significantly lower for positive control compared with negative control group. These results agree with those of Malasanos and Stacpoole (1991) who found that hypertriglyceridemia is most commonly associated with impaired glucose tolerance. Also Berger and Alford (2009) reported that a combination of excessive ingestion of caffeine- and taurine-containing energy drinks and strenuous physical activity can produce myocardial ischaemia by inducing coronary vasospasm. Data showed total (LDLc/HDLc) and (HDLc/total cholesterol) ratios as atherogenic indices for normal and hyperglycemic rats fed on high energy drink. The lower of HDLc/total cholesterol ratio reflect the increased risk of Coronary Heart Disease (CHD), meanwhile amplified of LDLc/HDLc ratio indicated the enlarged risk of CHD. It is obvious to notice that LDLc/HDLc ratio increased gradually when the level of HED increased as shown in Fig. 2. The same trend was observed for HDLc/total cholesterol ratios, the same levels showed the reduction of HDLc/cholesterol ratio compared with the negative control. In this respect Aviram and Fuhrman (1998) indicated that cholesterol and LDLc are the main contributor with incidence of CHD while HDLc levels are inversely related to CHD incidence. We can concluded that rats which received HED showed increase initiation of atherogenic indices than control-ve Heart Rate (HR) increased 5-7 beats/min and Systolic Blood Pressure (SBP) increased 10 mm Hg after energy drink consumption (Steinke *et al*., 2009). Such results were in agreement with that of Ragsdale *et al*. (2010) reported that energy drink consumption has been anecdotally linked to the development of adverse cardiovascular effects in consumers, although clinical trials to support this link are lacking. These findings suggest that consumption ameliorates changes in blood pressure during stressful experiences and increases the participants' pain tolerance. Another study by Libuda and Kersting (2009) showed the replacement of soft drinks and other sugar-containing beverages such as fruit juices by no caloric alternatives seems to be a promising approach for the prevention of overweight in childhood and adolescence. However, as the cause of overweight and obesity is multifactorial, the limitation of soft drink consumption needs to be incorporated in a complex strategy for obesity prevention.

REFERENCES

AOAC, 1975. Official Methods of Analysis. Association of the Official Analytical Chemists. 12th Edn., Washington, D.C.

Allain, C.C., 1974. Cholesterol enzymatic colorimetric method. J. Clin. Chem., 20: 470.

Aviram, M. and B. Fuhrman,1998. LDL oxidation by arterial wall macrophages depends on the oxidative status in the lipoprotein and in the cells: role of prooxidants vs. antioxidants. Mol. Cell Biochem. J., 188: 149-159.

Beck, T.W., T.J. Housh, R.J. Schmidt, G.O. Johnson, D.J. Housh, J.W. Coburn and M.H. Malek, 2006. The acute effects of a caffeine-containing supplement on strength, muscular endurance and anaerobic capabilities. J. Strength Cond. Res., 20: 506-510.

Berger, A.J. and K. Alford, 2009. Cardiac arrest in a young man following excess consumption of caffeinated energy drinks. Med. J., 190: 41-43.

Bichler, A., A. Swenson and M.A. Harris, 2006. Combination of caffeine and taurine has not effect on short term memory but induces changes in heart rate and mean arterial blood pressure. J. Amino Acids., 31: 471-476.

Bleich, S., D. Cutler, C. Murray and A. Adams, 2008. Why is the developed world obese? Annu. Rev. Public Health J., 29: 273-295.

Bleich, S.N., Y.C. Wang, Y. Wang and S.L. Gortmaker, 2009. Increasing consumption of sugar-sweetened beverages among US adults: 1988-1994 to 1999-2004. Am. J. Clin Nutr., 89: 372-381.

Boyle, M. and V.D. Castillo, 2006. Monster on the loose. J. Fortune., 154: 116-122.

Carleton, H., 1976. Histopathological Technique. 4th. Edn., London. Oxford University Press. New York.

Carrillo, J.A. and J. Benitez, 2000. Clinically significant pharmacokinetic interactions between dietary caffeine and medications. J. Clin. Pharmacokinet, 39: 127-153.

Chapman, D.G., R. Castilla and J.A. Campbell, 1959. Evaluation of protein in food I: A method for the determination of protein efficiency ratio. Can. J. Biochem. Physiol., 37: 679-686.

Desai, A. and M. Bhide, 1985. Hypoglycemic effect of Hanitonia suavcolens. Ind. J. Med., 81: 86-91.

Forbes, S.C., D.G. Candow, J.P. Little, C. Magnus and P.D. Chilibeck, 2007. Effect of red bull energy drink on repeated wingate cycle performance and bench-press muscle endurance. J. Sport Nutr. Exerc. Metab., 17: 433-444.

Friedewald, W.T., 1972. Determination of HDL. Clin. Chem., 18: 499.

Gordon, T. and M. Amer, 1977. Determination of HDL. J. Med., 62: 707.

Haussament, T.U., 1977. Determination of alkaline phosphates. Clin. Chem. Acta, 35: 271-273.

Header, E.A., 2006. The effects of daily oral feeding of curry and mustard on serum glucose, serum lipids and liver and kidney functions in diabetic rats. 9th Scientific Conference of Home Economics, Helwan University, Egypt, (18-19 April).

Kerrigan, S. and T. Lindsey, 2005. Fatal caffeine overdose: Two case reports. J. Forensic Sci. Int., 153: 67-69.

Lee, R. and D. Nieman, 1996. Nutritional Assessment. 2nd Edn., Mosby, Missouri, USA.

Lee, S.J., R. Hudson, K. Kilpatrick, T.E. Graham and R. Ross, 2005. Caffeine ingestion is associated with reductions in glucose uptake independent of obesity and Type 2 diabetes before and after exercise training. J. Diabetes Care, 28: 566-572.

Libuda, L. and M. Kersting, 2009. Soft drinks and body weight development in childhood: Is there a relationship? Curr. Opin. Clin. Nutr. Metab. Care J., 12: 596-600.

Malasanos, T.H. and P.W. Stacpoole, 1991. Biological effects of omega-3 fatty acids in diabetes mellitus. Diabetic Care, 14: 1160-1179.

Malik, V.S., M.B. Schulze and F.B. Hu, 2006. Intake of sugar-sweetened beverages and weight gain: A systematic review. Am. J. Clin. Nutr., 84: 274-288.

Mohamed Hala, A.W., 2004. Biological Effect of Some Legumes and Fruits on Hyperglycemic Rats. M.Sc. Thesis, Dept. of Nutrition and Food Science, Faculty of Home Economics, Minufiya University, pp: 99-105.

Mohamed, S.M., 2000. Head of cabbage, cauliflower and lettuce reduce blood glucose among diabetic rats. J. Home Econ.-Minufiya Univ., 11: 163-179.

Nation Diabetes Data Group (NDDG), 1994. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes, 28: 1039-1057.

Ogden, C.L., M.D. Carroll, L.R. Curtin, M.A. McDowell, C.J. Tabak and K.M. Flegal, 2006. Prevalence of overweight and obesity in the United States, 1999-2004. JAMA; 295: 1549-1555.

Pronsky, Z.M., 1997. Food Medication Interactions. 10. Crowe JP, editor. Pottstown, pp: 274.

Ragsdale, F.R., T.D. Gronli, N. Batool, N. Haight, A. Mehaffey, E.C. McMahon, T.W. Nalli, C.M. Mannello, C.J. Sell, P.J. McCann, G.M. Kastello, T. Hooks and T. Wilson, 2010. Effect of Red Bull energy drink on cardiovascular and renal function. J. Amino Acids., 38: 1193-1200.

Reeves, P.G., F.H. Nielsen and G.C. Fahmy, 1993. Reported of the American Institute of Nutrition Adhoc Writing Committee on the reformulation of the AIN-76 A Rodent diet. J. Nutr., 123: 1939-1951.

Reissig, C.J., E.C. Strain and R.R. Griffiths, 2009. Caffeinated energy drinks - a growing problem. Drug Alcohol. Depend., 99: 1-10.

Reitman, S. and S. Frankel, 1957. Determination of serum glutamic oxaloacetic transaminase and serum pyruvic transaminase. Am. J. Chem. Path., 28: 56-68.

Riesenhuber, A., M. Boehm, M. Posch and C. Aufricht, 2006. Diuretic potential of energy drinks. J. Amino Acids, 31: 81-83.

Scholey, A.B. and D.O. Kennedy, 2004. Cognitive and physiological effects of an energy drink: An evaluation of the whole drink and of glucose, caffeine and herbal flavoring fractions. J. Psychopharmacol. (Berl), 176: 320-330.

Schulze, M.B., J.E. Manson and D.S. Ludwig, 2004. Sugar-sweetened beverages, weight gain and incidence of type-2 diabetes in young and middle-aged women. JAMA, 292: 927-934.

Shoukry and A.E. Rhab, 2006. Biological Studies on Some Vegetables as Consumed by Diabetic Rats. M.Sc. Thesis, Dept. of Nutrition and Food Science, Faculty of Home Economics, Minufiya University, pp: 74-81.

SPSS, 2008. Statistical Package for Social Science, Computer Software, IBM, SPSS Ver. 16.0 in 2008., SPSS Company, London, UK.

Smit, H.J., M.L. Grady, Y.E. Finnegan, S.A. Hughes, J.R. Cotton and P.J. Rogers, 2006. Role of familiarity on effects of caffeine- and glucose-containing soft drinks. J. Physiol. Behav., 87: 287-297.

Steinke, L., D.E. Lanfear, V. Dhanapal and J.S. Kalus, 2009. Effect of energy drink consumption on hemodynamic and electrocardiographic parameters in healthy young adults. Ann. Pharmacother. J., 43: 596-602.

Trinder, P., 1969. Determination of triglycerides. Ann. Clin. Biochem., 6: 24-27.

Van den Eynde, F., P.C. Van Baelen, M. Portzky and K. Audenaert, 2008. The effects of energy drinks on cognitive performance. J. Tijdschr Psychiatr., 50: 273-281.

Wang, Y. and M.A. Beydoun, 2007. The obesity epidemic in the United States-gender, age, socioeconomic, racial/ethnic and geographic characteristics: A systematic review and meta-regression analysis. J. Epidemiol. Rev., 29: 6-28.

Young, D. and L. Pestaner, 1975. Determination of triglycerides. Germany J. Clin. Chem., 21: 25.