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## Histological and Physiological Studies on the Long-term Effect of Different Concentrations of Energy Drink (Tiger) on the Renal and Hepatic Systems of Young Mice

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### Abstract:

The present study aims to investigate the long-term histopathological, and physiological effects of different concentrations of a commercially available energy drink (Tiger) on liver and kidney of young mice. Sixteen Balb/c male mice, 6-week old, were divided into 4 groups (n=4). Two groups consumed the energy drink at a concentration of 28µl energy drink/ml water. One group were killed after 10 days (T1), another group were killed after 20 days (T2). Other group of mice consumed the energy drink at a final concentration of 14µl/ml for 20 days (T3). The last group was provided only with water and served as control. Mice of all groups drank around 3 ml per day. The histopathological study on liver of treated groups showed many changes such as inflammatory cells infiltration and aggregation with hepatocytes necrosis, some of these necrosis replaced by RBCs and inflammatory cells, while the pathohistological changes in kidney of treated groups limited to aggregation of RBCs and inflammatory cells between renal tubules which expressed vacuolar degeneration. These changes based on elevated liver function enzymes (Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT) and Alkaline phosphatase (ALP)) and blood urea and creatinine. These changes were more in the T2 groups, so it could be concluded that long term of energy drink consuming effect histopathologically and physiologically on kidney and liver of young mice depending on its concentration and period of consuming.

**Key words:** Energy Drinks EDs, Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT) and Alkaline phosphatase (ALP), kidney, liver

### Introduction:

Energy drinks are a group of non-alcoholic, often lightly carbonated beverages used to provide energy, diminishing sleep needs and keeping, and providing cognitive and enhancement of mood (1). Young people widely use it during study, playing sports, and long distance driving (2). Energy drinks consumption has increased worldwide since 1987 when their appearance on the market (3).

Energy drinks commonly include caffeine, taurine and other amino acid such as carnitine and creatine, simple sugars (glucose and fructose), herbal supplements like ginseng and ginkgo biloba, maltodextrin, inositol, glucuronolactone, and vitamins B complex (4). Additives such as Guarana, Merba, Cocoa, and cola can increase the caffeine content of energy drinks without the knowledge of consumers. Manufacturers of these products are not required to include the caffeine content of these herbal supplements in nutritional information.

Different brands of energy drinks contain caffeine ranging from 50mg to 550mg per can or bottle (5).

This higher caffeine concentration may result in poisoning and some its containing which highly stimulating properties may results overdose (6). In addition, consumption dosage of energy drinks has been associated with strokes, seizures (7), and effect of Gonadotoxic (8).

Caffeine, the main component of energy drinks, is associated with diuresis and the balance of electrolyte in fluid. Taurine is associated to detoxification and bile acid conjugation. Some studies show that the caffeinated energy drinks affect liver cells and increase creatinin (9, 10), these drinks affect liver enzymes such as ALT, AST, and ALP (11,12). Other researcher reported that the energy drink effects on renal functions (13).

Other studies have indicated that consuming of energy drinks have affected blood chemistry and the activities of liver enzyme. On the other hand, Ebuehi, 2011 reported that there were

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no obvious histopathological abnormalities of the brain and liver (14), while Khayyat reported histopathological abnormalities of liver (15). There is a previous research that showed histopathological abnormalities of many regions in brain (16). So the present study aims to investigate the long-term histopathological, and physiological effects of different concentrations of a commercially available energy drink on liver and kidney of young mice.

### Materials and Methods:

Sixteen Balb/c male mice, 6 -week old, were divided into 4 groups (n=4). One group consumed the energy drink (Tiger) at a concentration of 28 $\mu$ l energy drink/ml water, and the animals were killed after 10 days (T1). Another group consumed the energy drink at the same concentration but during 20 days (T2). A third group of mice consumed the energy drink at a final concentration of 14 $\mu$ l/ml for 20 days (T3). The fourth group was provided only with water and served as control. Mice of all groups drank around 3 ml per day.

The mice were weighed and killed by cervical distraction at different times after energy drink consumption. Blood was collected from eyes in sterile tubes. Body/organs index was calculated using the equation (bodyweight /organ weight). Liver and kidney were collected, weighed and put

in 10% formalin solution, processed by standard procedures. Sections of paraffin-embedded tissues were stained with heamatoxyline and eosin and examined by light microscopy (17). Blood samples were centrifuged for 5min at 5000rpm. The serum was separated from blood and used to determine blood urea, creatinine, GPT, GOT, and ALP levels by Bu, craetinine, GPT, GOT, and ALP kits from Randox. All the experiments were done in Department of Biology, Collage of Science, Mustansiriyah University.

Results are expressed as mean  $\pm$  standard Error (M $\pm$ SE). Data were analyzed by one-way analysis of variance (ANOVA) followed by Fisher's test for multiple comparisons, using Statview version 5.0. Differences were considered significant when  $p < 0.05$ .

### Results:

#### A. Body and Organs Weight:

There were no significantly differences among body weight, organs weight, and body/organs index of all groups except body weight after energy drink consuming of treated 2 and 3 groups compared to control while there were no differences between their body weight after and before consuming (Table 1)

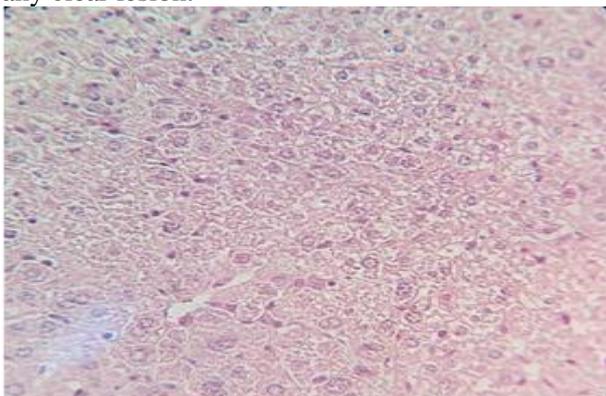
**Table 1. Body weight, organs weight, and organ/body weight index (M $\pm$ SE) of treated and control groups**

Groups	B.weight before (g)	B.weight after (g)	Liver weight (g)	Kidney weight (g)	Liver/Body weight index	Kidney/Body weight index
Control	24 $\pm$ 1.1	24.8 $\pm$ 0.6	1.1 $\pm$ 0.09	0.42 $\pm$ 0.02	0.046 $\pm$ 0.004	0.017 $\pm$ 0.009
Treated 1	24.5 $\pm$ 0.9	26.5 $\pm$ 0.6	1.0 $\pm$ 0.07	0.26 $\pm$ 0.03	0.038 $\pm$ 0.003	0.010 $\pm$ 0.001
Treated 2	27.7 $\pm$ 1.5	28.7 $\pm$ 1.3*	1.3 $\pm$ 0.14	0.50 $\pm$ 0.01	0.044 $\pm$ 0.003	0.017 $\pm$ 0.001
Treated 3	26.5 $\pm$ 1.3	28.0 $\pm$ 1.2*	1.1 $\pm$ 0.09	0.52 $\pm$ 0.03	0.041 $\pm$ 0.002	0.019 $\pm$ 0.001

\*Significant difference between treated and control groups

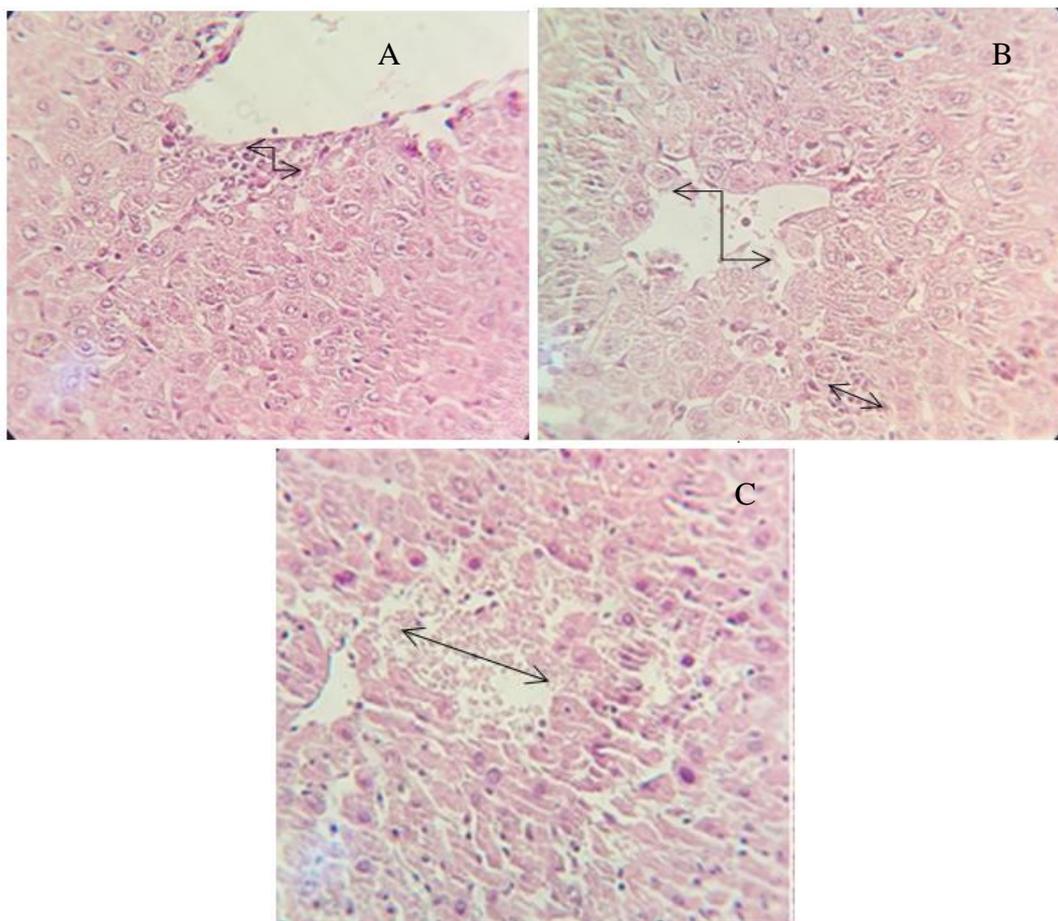
#### B. Histopathological Study:

Figure1 shows histological section in the liver of control animal which shows normal liver without any clear lesion.



**Figure 1. Histological section in liver of normal animal shows no clear lesion (H&E stain 400X)**

The histopathological study on liver of treated groups showed many changes compared to normal section in control group. In (T1) group, consumed the concentrated energy drink for 10 days, the sections show inflammatory cells infiltration and aggregated in small area around blood vessels (Fig. 2A) and in liver parenchyma with hepatocyt's necrosis (Fig. 2B) some of these necrosis replaced by RBCs and inflammatory cells (Fig. 2C).



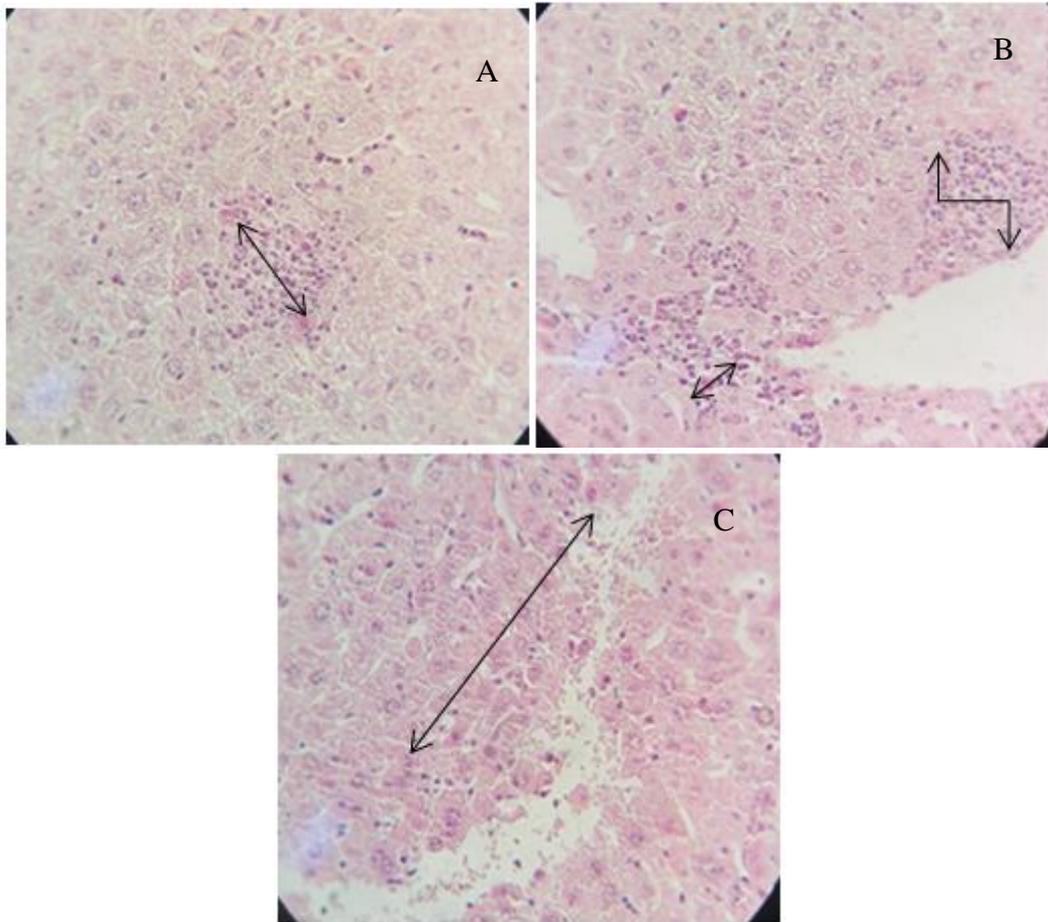
**Figure 2. Histological section in liver of (T1) group (H&E stain 400X), (A): shows inflammatory cells infiltration and aggregation around blood vessels ,(B): shows necrosis and inflammatory cells infiltration of liver parenchyma ,(C): RBCs and inflammatory cells replacement necrotic area of hepatocytes**

Figure 3 shows many areas in liver histological sections of (T2) group, consumed the concentrated energy drink for 20 days, has infiltration and aggregation of inflammatory cells within liver parenchyma (Fig. 3A), around blood vessels and in sinusoids (Fig. 3B) hepatocytes necrotic area filled with RBCs and inflammatory cells (Fig. 3C).

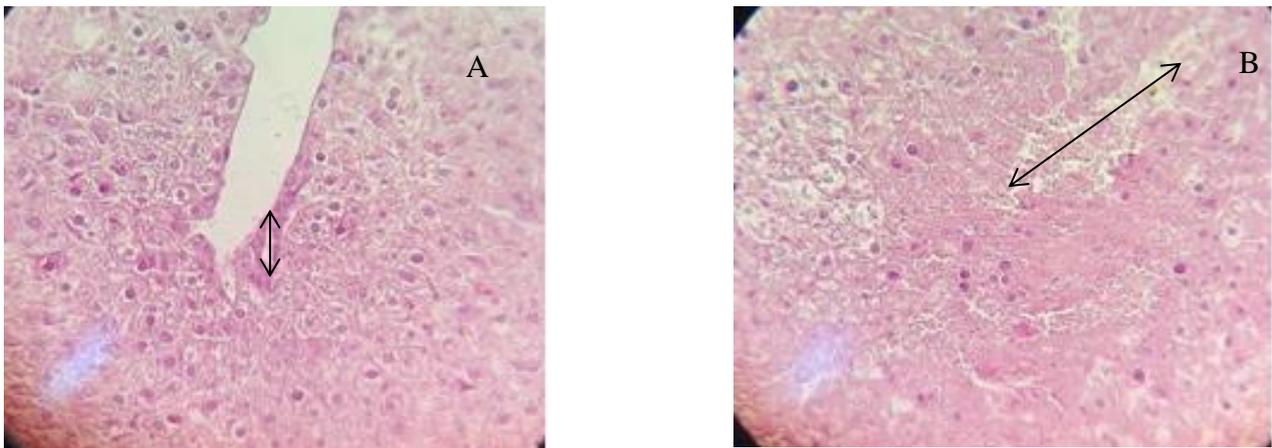
The histopathological changes in the liver section of (T3) group, consumed the diluted energy drink for 20 days, were less than other treated groups such as infiltration inflammatory cells in very small area around blood vessels (Fig. 4A) but

there was no aggregation in liver parenchyma and there were small areas of hepatocytes necrotic replaced by RBCs and inflammatory cells (Fig. 4B)

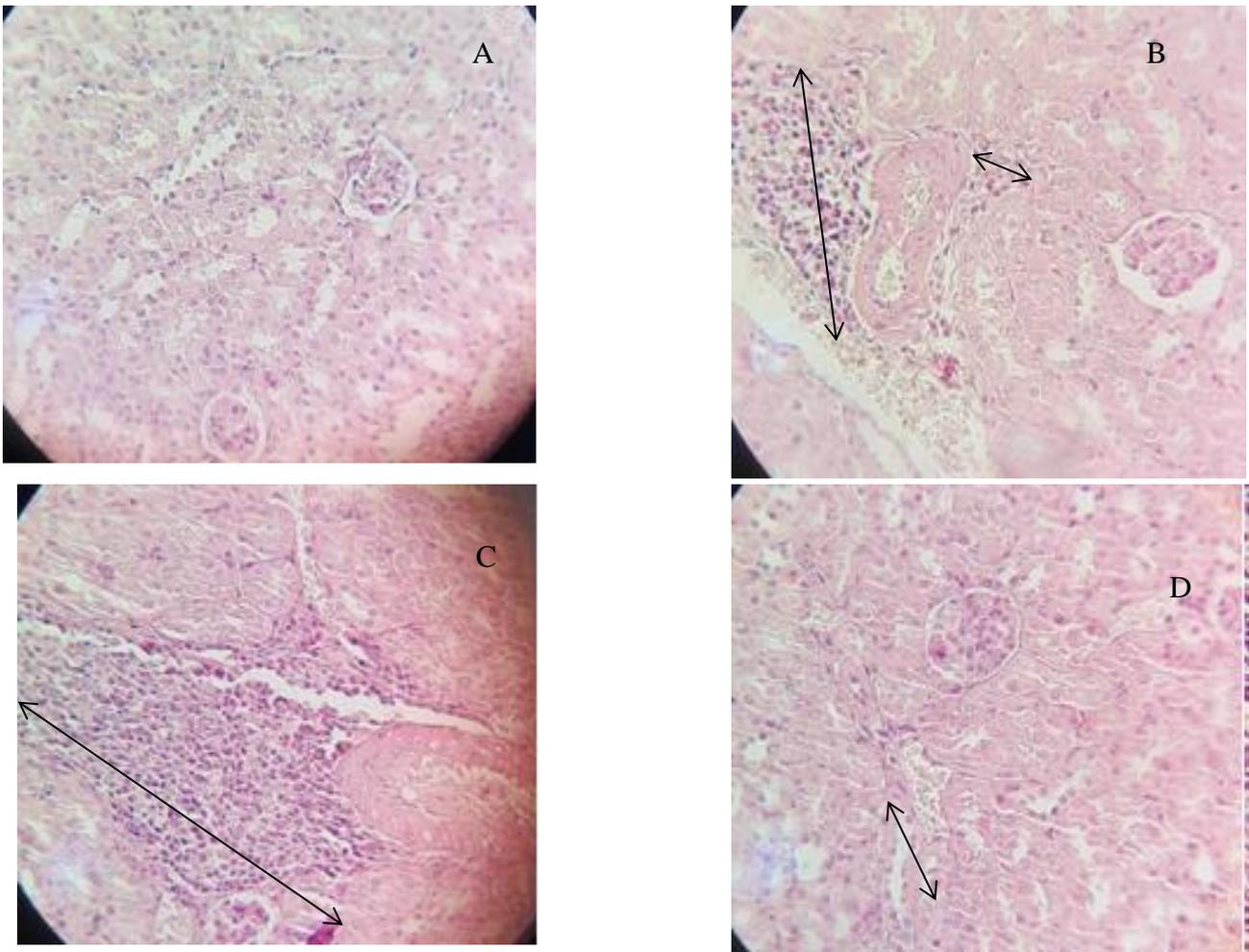
The histopathological changes in kidney of treated groups limited to aggregation of RBCs and inflammatory cells among renal tubules which expressed vacuolar degeneration. The biggest aggregation area was in the (T2) group (Fig. 5C), the bigger was in the (T1) (Fig. 5B), and the smallest was in the (T3) group (Fig. 5D) compared to normal section which shows no clear lesion in the control group (Fig. 5A)



**Figure 3: Histological section in liver of (T2) group (H&E stain 400X) (A): shows inflammatory cells aggregation in liver parenchyma with megakaryocytes in the sinusoids , (B): shows inflammatory cells infiltration and aggregation around blood vessels and in sinusoids , (C): RBCs and inflammatory cells replacement necrotic area of hepatocytes**



**Figure 4. Histological section in liver of (T3) group (H&E stain 400X), (A): shows inflammatory cells infiltration around blood vessels, (B): RBCs and inflammatory cells replacement necrotic area of hepatocytes**



**Figure 5. Histological section in liver of all groups (H&E stain 400X), (A) in control, (B) in T1, (C) in T2, and (C) in T3 groups, (A): Histopathologica section in the kidney of normal animal shows no clear lesions, (B): shows RBCs and inflammatory cells between renal tubules that expressed vacuolar degeneration,(C): shows of RBCs and infammatory cells between renal tubules that expressed vacuolar degeneration,(D): shows RBCs and inflammatory cells between renal tubules that expressed vacuolar degeneration**

The physiological study of liver and kidney included their enzymes determination. GOT, GPT, and Alkaline phosphate levels were also higher in the treated groups compared to control group but it

reached to significant higher ( $P < 0.05$ ) GOT and GPT only in the T2 group and Alkaline phosphate in T2 and T3 groups (Fig. 6A,B,&C)

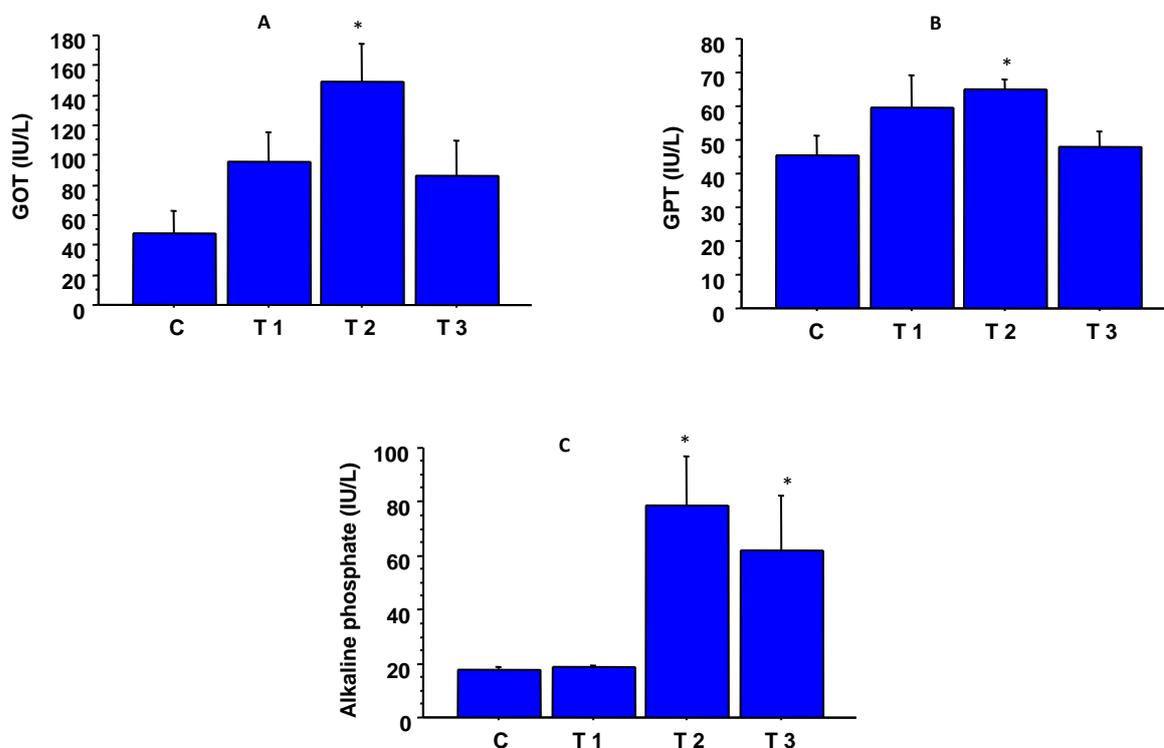


Figure 6. GOT, GPT, and Alkaline phosphate levels in treated and control groups, \*significantly difference between treated and control groups

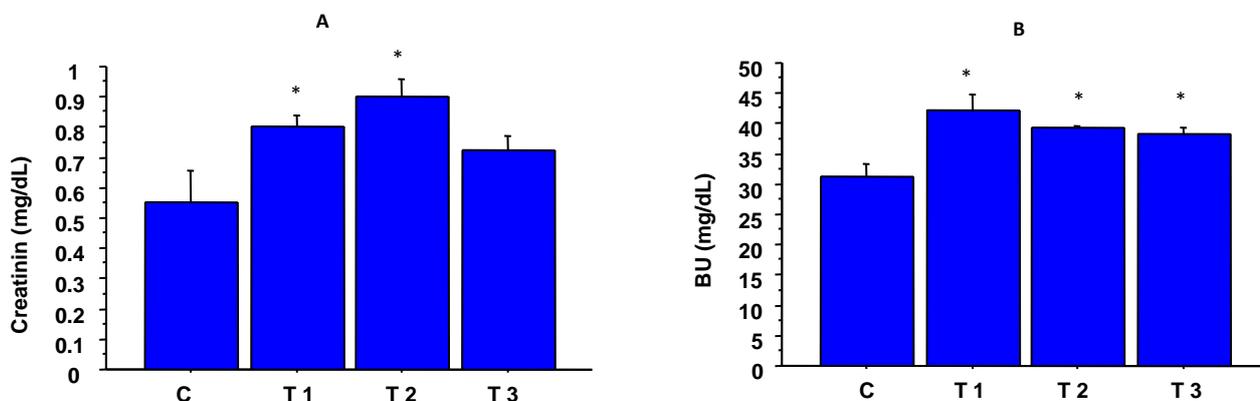


Figure 7. Creatinine and blood urea levels in treated and control groups, \*significantly difference between treated and control groups

**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 (18). On the other hand, it is well established that taurine is associated with bile acids and helped fat digest (19). 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. These

could be attributed to degenerative changes within the liver cells (15,20).

On the other hand, many works studied the ultrastructural alterations of hepatocyt of animals that consumed energy drinks and reported presence dilatation and fragmentation of rough endoplasmic reticulum cisternae (21), which can damage hepatocytes (22-24). In addition, Balaban (2005) indicated deterioration in mitochondrial function because of disruption in mitochondrial structure (25). The study of Mubarak showed irregular outlines and pyknosis and numerous mitotic figures in hepatocytic nuclei (20). Mubarak (2012) attributed these changes to preservatives added to energy drinks such as sodium benzoate, and to the caffeine toxic work (20). These changes could lead

to hepatocyt necrosis which indicated in this present study.

The present study also showed elevation in the liver function enzymes GOT, GPT, and ALP sera levels in the mice consumed energy drink which agree with many studies (14, 26, 27).

Typically, energy drinks contain 80-141 mg of caffeine at 8 oz, equivalent to five ounces of coffee or two cans of 12 ounces of caffeinated soft drinks (28). This higher caffeine concentration caused elevation in GOT and GPT sera levels of rats (29), while other studies reported that the caffeine caused decrease in GOT level (30, 31).

As mentioned above, the combination of caffeine and taurine the containing of energy drinks excessive ingestion can produce ischaemia of myocardial by inducing coronary vasospasm (18). On the other hand, the combination of caffeine and sugar elevate the blood pressure (32). Greene (2014) reported that the acute renal failure occur in the context of ingestion of large quantities of energy drink or with concomitant alcohol (13). These evidences can explain the histopathological changes found in this work such as RBCs and leukocytes infiltration and aggregation between renal tubules which could express vacuolar degeneration. This finding is based on the results of creatinin and blood urea levels which elevate in the serum of mice consumed energy drink compared to control groups.

The toxic effect of caffeine may depend on certain physiological or pathological conditions such as dose, chronic pre-exposure, enzymatic genetic markers, and associated with drug consumption (33). This evidences can explain the less histopathological and physiological changes in the T3 group which consumed diluted concentration of energy drink compared to T2 group which consumed concentrated energy drink for the same period and explain the different changes between treated 2 and treated 1 groups which consumed the same concentration of energy drink but in different periods.

### Conclusion:

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

### Conflicts of Interest: None.

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## دراسة نسيجية وفسلجية حول تأثير تناول تراكيز مختلفة لمشروبات الطاقة ولمدة طويلة على الجهاز البولي والكبد في الفئران صغيرة العمر

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### الخلاصة:

هذه الدراسة تهدف الى التحقق من التأثيرات الفسلجية والنسيجية المرضية لاستعمال طويل الامد لتراكيز مختلفة من مشروبات الطاقة الاكثر شيوعا لدينا على الكبد والكلى لفئران صغيرة السن. فئران بأعمار ست اسابيع من نوع البينو تم تقسيمها الى اربعة مجاميع. المجموعة الاولى تناولت مشروب الطاقة بتركيز 28 مايكرليتر/مللتر ماء، وتم قتلها بعد عشرة ايام (T1). المجموعة الاخرى تناولت مشروب الطاقة لنفس التركيز لكن تم قتلها بعد 20 يوم (T2). المجموعة الثالثة تناولت مشروب الطاقة بتركيز 14 مايكرليتر/مللتر ماء ولمدة 20 يوم (T4). المجموعة الرابعة تم تجهيزها بالماء فقط لتكون مجموعة سيطرة. تم قتل الفئران و تجميع عينات الدم و اعضاء الفئران (الكبد والكلى). الدراسة النسيجية المرضية لمقاطع كبد الفئران التي تناولت مشروب الطاقة اظهرت تغيرات كثيرة كترشح خلايا التهابية وتجمعها في الكبد مع تنكز لبعض المناطق وبعضها قد امتلأت بخلايا الدم الحمراء والالتهابية بينما كانت التغيرات في كلى المجاميع التي تناولت مشروبات الطاقة محدودة حيث اقتصر على تجمعات لخلايا الدم الحراء والالتهابية بين الانبيبات البولية والذي يعرب عن تنكس وعائي. هذه التغيرات اتفقت مع ما وجد من ارتفاع بالانزيمات الخاصة بوظائف الكبد (GOT، GPT، و ALP) اضافته الى اليوريا والكرياتنين الخاصة بوظائف الكلية. هذه التغيرات كانت متزايدة في مجموعة T2 ولهذا يمكننا ان نستنتج ان تناول مشروبات الطاقة لمدة طويلة تؤثر نسيجيا و فسلجيا على الكلية والكبد للفئران صغيرة السن وهذا التأثير يعتمد على تركيز مشروب الطاقة ومدة تعاطيه.

**الكلمات المفتاحية:** مشروب الطاقة، انزيم ناقلة امين الغلوتاميك للاكسالواسيتيك، انزيم ناقلة امين الغلوتاميك للبايروفيك، انزيم ناقلة امين الاسبارتات، الكلية، الكبد.