

Study of Physiological and Histological Changes in Male Rats (*Rattus norvegicus*) After Carbonated and Energy Beverages Drinking

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Abstract

This study was conducted to detect the effect of Pepsi cola and Tiger beverages drinking in some physiological parameters and histopathological changes in male rats , thirty two male rats were used which divided into four groups , for each group included eight animals (n=8) the first group administrated physiological saline as control group , the second group administrated (1mL) Pepsi cola , the third group administrated two doses of Pepsi cola by (1mL) in each dose and the duration between these two doses was 4 hours while the fourth group administrated (1mL) Tiger beverage. The results of present study showed a significant increasing ($P \leq 0.05$) in body weight, sperm malformations, urea concentration, glucose level and triglycerides in treated groups by Pepsi and Tiger beverages compared with control group. Also, the results found a significant decreasing ($P \leq 0.05$) in the sperm count , ALT , AST, ALP , albumin level and total protein of treated groups (second , third and fourth groups) compared with control group , While observed non-significant increasing in creatinine concentration and cholesterol level of treated groups (second , third and fourth groups) compared with control group .

The results showed that treatment of male rats by Pepsi and Tiger beverages caused histological changes in testes such as necrosis of epithelia , destroyed and increasing the thickness of interstitial tissue , congestion and reduction of spermatocytes . In kidneys that included congestion , infiltration of inflammatory cells , reduce , absence and death of glomeruli , enlargement of Bowman's space and hemorrhage .

While in liver that comprised inflammation, congestion, enlargement of sinusoids, hypertrophy of nuclei for hepatocytes and hemorrhage.

Keywords: Carbonated beverages, Sperm, Histological changes.

Introduction

Carbonated beverages are term refers to all beverages that contain carbon dioxide . Soft drinks are carbonated non-alcoholic beverages with a sweet bubbly refreshing taste prepare by pressuring carbon dioxide in waters (92%) which have special specifications added to their sucrose , articles including acids , colorings , flavors and preservatives that to be ready for human consumption , Pepsi cola is one of carbonated beverages which discovered by Caleb Brand ham in 1898 (Philip and Ashurst, 2005) . In the last three decades , the consumption by children and adolescents of these beverages have increased dramatically (Saad, 2014) . At least one carbonated beverage is consumed by 66% of children and 77% of adolescents daily (Andreyeva *et al.*, 2011) . Including adult and adolescent, male have been found to consume more carbonated beverages than female (Ogden *et al.*, 2011) . Carbonated beverages are contain high compounds of phosphoric acid which cause losing of calcium for body led to osteoporosis, and increase glucose levels that stimulate pancreas to excrete insulin causing diabetes mellitus (Morengal *et al.*, 2012) .

Energy beverages are carbonated beverages direct or indirectly added some of stimulants such as caffeine by addition extracts of plants as Guarana that have active effect on central nervous system (Oteri *et al.*, 2007) . The purpose of using this drinks for granting enormous metabolic energy and mental toughness because their contents of stimulants proteins, carbohydrates , amino acids , mineral, vitamins, acids and other components (Malinauskas *et al.*, 2007) .There are a relationship between excessive consumption of these beverages and harmful effects including loss of particularly enamel, insomnia , headache , vomiting and arrhythmia (Kitchens and Owens, 2007) .

The aim of the present study was to investigate the possible effects of carbonated and energy beverages consumption on the physiological parameters and histological changes in male rats.

Materials and Methods

Samples Collection

Cans of Pepsi cola-type beverage and Tiger-type energy beverage were obtained from local markets in Thi-Qar province daily for administration of animals.

Laboratory Animals and Carbonated Beverages Administration

This study used thirty two adult albino rats of male *Rattus norvegicus* (12-14) weeks old, with average weight of (240-260) grams. The rats were obtained and maintained in the animal house of the biology department /college of education for pure sciences / Thi-Qar university /Iraq under standard circumstances (Al-Maliki, 2000). Animals were randomly divided into four groups. For each (8 for each group) as follows: First group control group which treated with a physiological normal saline of (0.9% NaCl) , the second group which treated with one dose Pepsi cola-type beverage by 1 mL /animal/day , the third group which treated with two doses of Pepsi cola-type beverage by 1 mL /animal/day in each dose and the duration between these two doses was 4 hours . While the fourth group was treated with Tiger -type energy beverage by 1mL /animal/day. All animals treated orally by stomach tube and the experiment lasted for thirty days.

Body

Weight

After the demising the period of experiment, each animal were weighted and the weight of treated groups compared with control group.

The Sperm Count

The experiment was done according to Soto (1983) as follows: The left epididymis was cut into many small pieces and these were put in test

tubes, 2 mL of buffered formalin salt (which consists of 5 grams of sodium bicarbonate and 100 mL formalin) was added for each tube then 0.1mL of eosin stain (5%) . These tubes were put in centrifuge (1500 cycle/minute) for 5 minutes. Sperms counted by heamocytometer .

Total of sperms = number of sperms in five squares $\times 10^4$

Sperm Malformations

The method of Wyrobek and Bruce (1975) was used to determination of percentage abnormalities .

The right epididymis was cut into many pieces and these were put in test tubes with 5mL (0.9%) physiological saline for 15 minutes . Then drops of resulted solution was spreading on the slide and dried . Five slides was done for each sample , All slides were stained by eosin stain (1%) for 10 minutes and dried . After that slides were washing by tab water then left to be dried . Serially , 100 sperms were counted for each sample (five slides) which divided into : normal sperms that possess head , middle piece and tail ,and abnormal sperms that lack anyone of their parts or they have morphological changes .

Biochemical Parameters

The end of experiment period , blood samples were collected by cardiac puncture in tubes without EDTA anti-coagulant for studying the biochemical parameters . Serum was obtained from separated of blood by centrifuge at 3000 rpm for 10 minutes and stored at (-20°C) . Kidney functions measurement included serum urea and creatinine concentration that were estimated by urea and creatinine kits (Biomerieux , Biolabo/France) respectively (Tietz, 1999 ;Wills and Savory, 1981) . Determination of liver enzymes included Alanine Transaminase (ALT) , Aspartate Transaminase (AST) by using colorimetric method kits (Atlas medical /England) (Reitman and Frankel, 1957) and Alkaline phosphatase (ALP) by ALP kit (Biomerieux/France) (Belfield and Goldberg, 1970) . According to enzymatic method kits (Biolabo/France) , cholesterol and triglycerides were estimated (Fossati and Prencipe, 1982 ; Allain *et al.*, 1974) . Glucose , albumin and total proteins were determined by using kits (Biocon/Germany and Biolabo/France)

respectively (Tietz, 1995; Doumas and Biggs, 1972) . All of above biochemical parameters were measured by spectrophotometer in college of sciences.

Histological Study

After ending up of period experiment on the thirty -first day , all animals were sacrificed by cervical dislocation . The organs (testes , kidney and liver) dissected out and fixed in formalin 10% buffered solution for routine histological techniques . The tissues were dehydrates in an ascending grade of alcoholic (ethanol) , cleared in xylene and embedded in paraffin wax . Serial sections of 5 micrometer thick were obtained using a rotary microtome . The deparaffinized sections were stained routinely with haematoxylin and eosin (H&E) (Bancroft and Gamble, 2008) .

Statistical Analysis

The data are presented as means \pm S.D and statistically analyzed using SPSS(Version 19) . Significance was set at the level of ($p\leq 0.05$) (George and Mallery, 2011) .

Results

The present results in table (1) indicated to a significant increasing ($p\leq 0.05$) in body weight and malformation sperms of groups that treated by carbonated and energy beverages compared with control group . These malformations included many changes in head and tail shape such as global head , quirky tail and lacking head and tail sperms (pictures 2-4) .While a significant decreasing ($p\leq 0.05$) observed in sperm count in treated groups compared with control group.

Group	Body weight (gram)	sperm count ($\times 10^4$)	Sperm malformations (%)
First group control group	268.70 \pm 10.89 ^a	208.16 \pm 8.72 ^a	4.00 \pm 2.28 ^a
Second group treated with Pepsi (One dose)	272.35 \pm 27.64 ^b	183.50 \pm 9.66 ^b	87.11 \pm 27.40 ^b

Third group treated with Pepsi (Two doses)	306.41± 13.30 ^b	152.16 ± 9.66 ^b	96.12±2.51 ^b
Fourth group treated with Tiger beverage	285.18 ± 9.90 ^b	129.66± 14.90 ^b	98.35 ± 1.02 ^b
Sig.	0.00	0.00	0.00

Table (1) : Effect of carbonated beverages in body weight , sperm count and malformations (n=8) (Mean ± Standard deviation) Different letters refer to significant difference(P≤ 0.05) compared with control group

Biochemical parameter

The results found a significant increasing (P≤0.05) in urea concentration and non-significant increasing in creatinine of treated groups compared with control group . A significant decreasing (P≤0.05) was observed in liver enzymes (ALT, AST and ALP) of treated groups compared with control group table (2) .

Table (3) demonstrated a significant increasing (P≤0.05) in sugar level and triglycerides , significant decreasing (P≤0.05) in albumin level and total protein while non-significant increasing in cholesterol level of treated groups compared with control group .

Group	Urea (mg/dL)	Creatinine (mg/dL)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
First group control group	45.00± 3.09 ^a	0.76± 0.08 ^a	23.00± 4.69 ^a	24.66± 4.32 ^a	143.33± 19.79 ^a
Second group treated with Pepsi (One dose)	49.00± 9.87 ^b	0.81± 0.07 ^a	11.66± 1.36 ^b	10.33± 1.50 ^b	88.66± 15.30 ^b
Third group treated with Pepsi (Two	61.16± 14.17 ^b	0.81± 0.09 ^a	9.00± 0.89 ^b	9.16± 0.98 ^b	71.50± 14.55 ^b

doses)					
Fourth group treated with Tiger beverage	61.16± 10.85 ^b	0.83± 0.08 ^a	8.33± 1.50 ^b	9.66± 1.50 ^b	55.61± 7.83 ^b
Sig.	0.02	0.56	0.00	0.00	0.00

Table (2) : Effect of carbonated beverages on kidney functions and liver enzymes (n=8) (Mean ± Standard deviation)

Different letters refer to significant difference(P≤ 0.05) compared with control group

Table (3) : Effect of carbonated beverages on biochemical parameters (n=8) (Mean ± Standard deviation)

Group	Sugar (mg/dL)	Albumin (gm/L)	Total protein (g/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)
First group control group	110.50± 8.21 ^a	36.16±0.98 ^a	4.83± 0.75 ^a	59.16±5.67 ^a	36.66±8.71 ^a
Second group treated with Pepsi (One dose)	178.33± 31.20 ^b	30.16±3.76 ^b	3.50± 0.54 ^b	72.33±21.88 ^a	46.50±9.26 ^b
Third group treated with Pepsi (Two doses)	190.16± 25.05 ^b	27.00±5.05 ^b	2.66± 0.81 ^b	78.83±9.19 ^a	54.16±8.63 ^b
Fourth group treated with Tiger beverage	288.66± 20.89 ^b	24.66±3.50 ^b	2.20± 0.83 ^b	77.33±15.92 ^a	51.16±7.60 ^b
Sig.	0.00	0.00	0.00	0.11	0.01

Different letters refer to significant difference(P≤ 0.05) compared with control group

Histopathological Changes of Carbonated and Energy Beverages

Histological examination of testes, kidneys and livers was revealed many damages in treated groups by carbonated and energy beverages such as necrosis of epithelia , destroyed and increasing the thickness of interstitial tissue , congestion and reduction of spermatocytes . In kidneys the results showed congestion , infiltration of inflammatory cells , reduce , absence and death of glomeruli , enlargement of Bowman's space and hemorrhage . Histological effects in livers were included inflammation , congestion , enlargement of sinusoids , hypertrophy of nuclei for hepatocytes and hemorrhage as follows (pictures 5-20) .

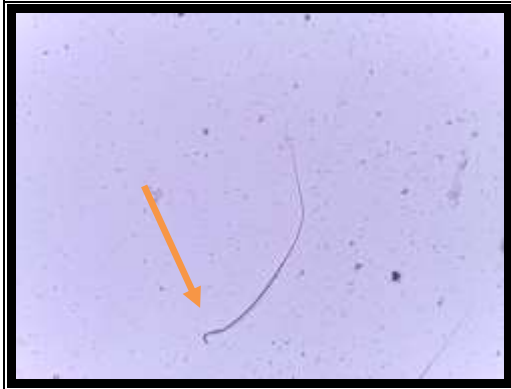


Figure (1) showed normal sperm of **control group** (Eosin Stain) (100 X) .



Figure (2) showed malformation sperm (global head) of **one dose of Pepsi beverage group** (Eosin Stain) (100 X).



Figure (3) showed abnormal sperm (quirky tail) of **two doses of Pepsi beverage group** (Eosin Stain) (100 X).



Figure (4) showed abnormal sperms of **energy beverage group**: lacking tail (A) lacking head (B) (Eosin Stain) (100 X) .

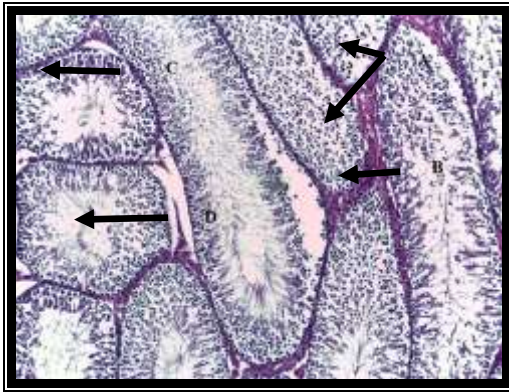


Figure (5) showed transverse section in testis of **control group** showing seminiferous tubules (A) interstitial tissue (B) spermatocytes (C) sperms (D) (H&E) (100 X) .

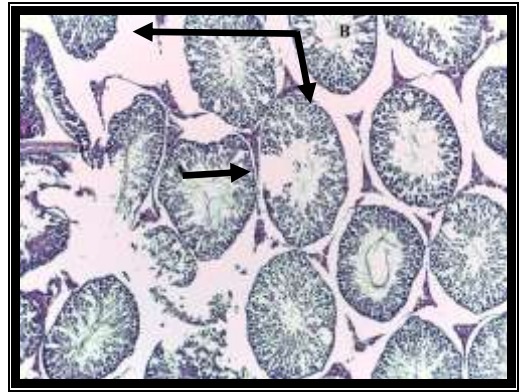


Figure (6) showed transverse section in testis of **one dose of Pepsi beverage group** showing necrosis of epithelial tubular (A) destroyed of interstitial tissue (B) (H&E) (100 X) .

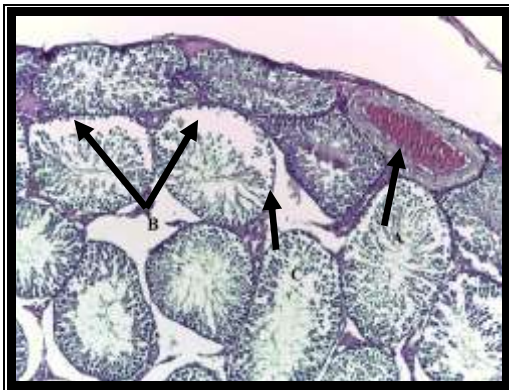


Figure (7) showed transverse section in testis of **one dose of Pepsi beverage group** showing congestion (A) reduction of spermatocytes (B) destroyed of interstitial tissue (C) (H&E) (100 X) .

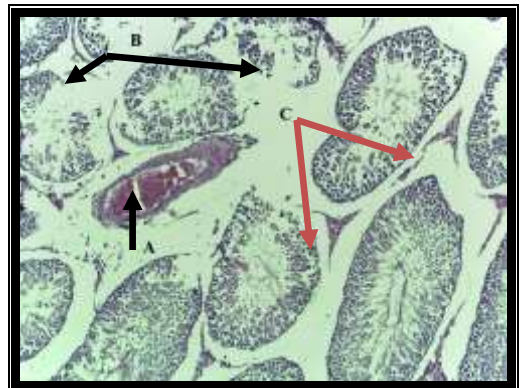


Figure (8) showed transverse section in testis of **two doses of Pepsi beverage group** showing congestion (A) necrosis (B) destroyed of interstitial tissue (C) (H&E) (100 X) .



Figure (9) showed transverse section in testis of **two doses of Pepsi beverage group** showing increasing thickness of connective tissue (A) (H&E) (100 X) .



Figure (10) showed transverse section in testis of **energy beverage group** showing reduction of spermatocytes (A) destroyed of interstitial tissue (B) (H&E) (100 X) .

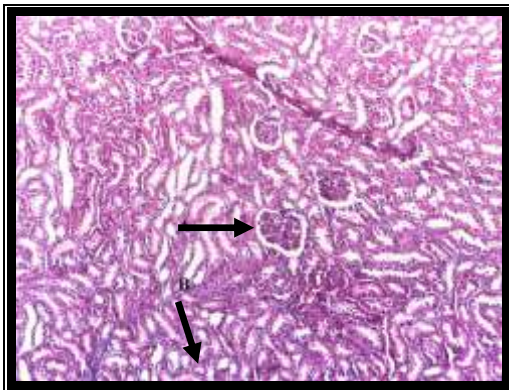


Figure (11) showed transverse section in the kidney of **control group** showing glomerulus (A) renal tubules (B) (H&E) (100 X) .

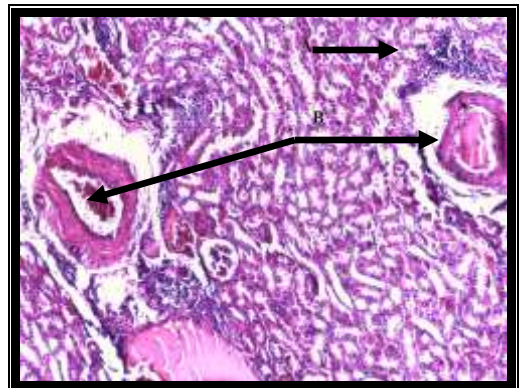


Figure (12) showed transverse section in the kidney of **one dose of Pepsi beverage group** showing infiltration of inflammatory cells (A) congestion (B) (H&E) (100 X) .

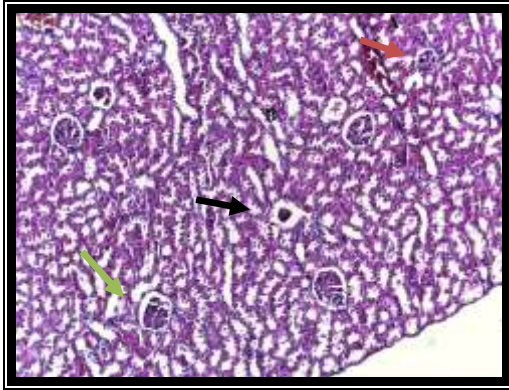


Figure (13) showed transverse section in the kidney of **one dose of Pepsi beverage group** showing atrophy of glomerulus (A) absence (B) enlargement of Bowman's space (C) (H&E) (100 X) .

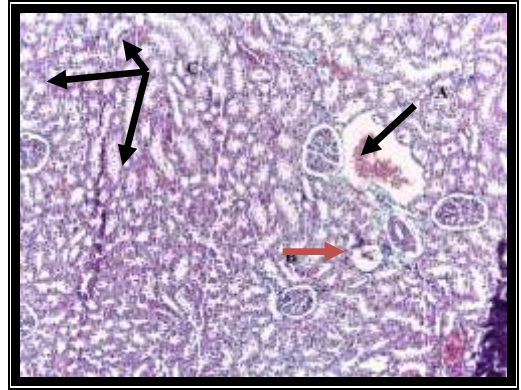


Figure (14) showed transverse section in the kidney of **two doses of Pepsi beverage group** showing congestion (A) absence of glomerulus (B) hemorrhage (C) (H&E) (100 X) .

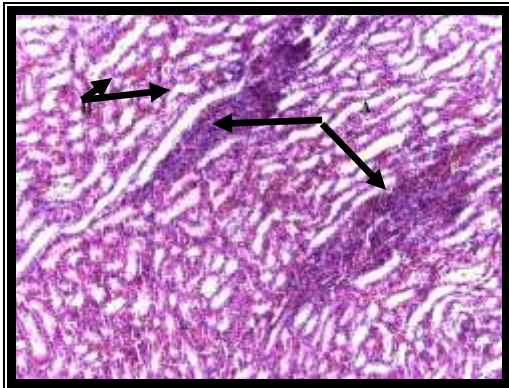


Figure (15) showed transverse section in the kidney of **energy beverage group** showing large inflammation (A) hemorrhage among renal tubules (B) (H&E) (100 X) .

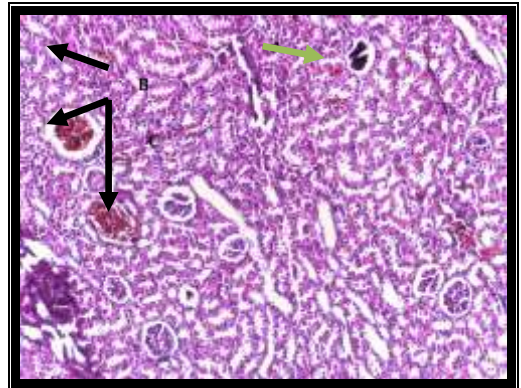


Figure (16) showed transverse section in the kidney of **energy beverage group** showing death of glomerulus (A) hemorrhage (B) congestion (C) (H&E) (100 X) .

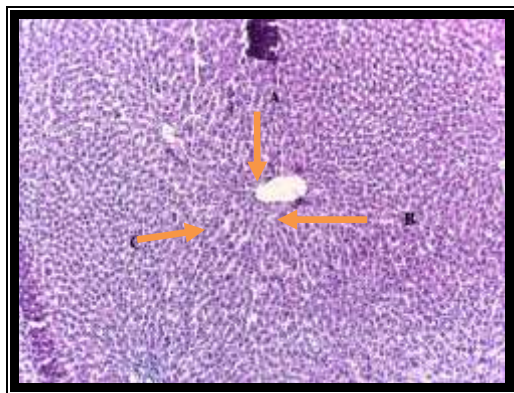


Figure (17) showed transverse section in the liver of **control group** showing central vein (A) hepatocytes (B) sinusoids (C) (H&E) (100X) .

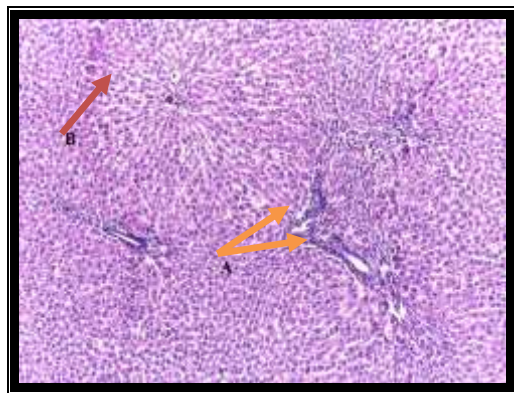


Figure (18) showed transverse section in the liver of **one dose of Pepsi beverage group** showing infiltration of inflammatory cells (A) enlargement of sinusoids (B) (H&E) (100X) .

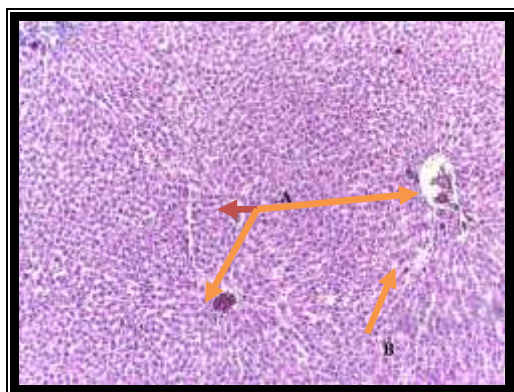


Figure (19) showed transverse section in the liver of **two doses of Pepsi beverage group** showing large congestion (A) enlargement of sinusoids (B) (H&E) (100X) .

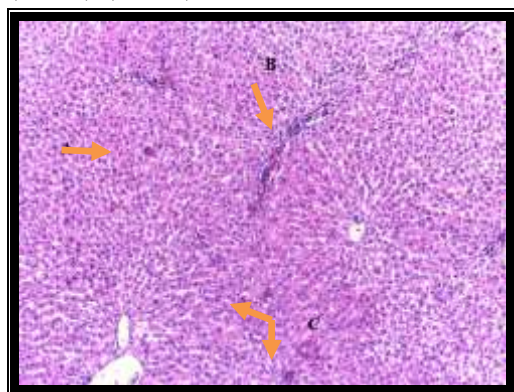


Figure (20) showed transverse section in the liver of **energy beverage group** showing hypertrophy of nucleus of hepatocyte (A) inflammation (B) hemorrhage (C) (H&E) (100X) .

Discussion

The results reported a significant increasing of body weight of treated groups , that occur clearly because soft drinks consumption, this agree with DiMeglio and Mattes (2000) showed a positive correlation between sugar-sweetened beverages consumption and increased body weight . Also , this result agreement with Swinburn *et al.* (2004) who showed excessive drinking of carbonated beverages causes high weight and obesity .

Significant decreasing in sperm count and significant increasing of malformations sperm were observed in treated groups , these may be regarded to oxidation and free radicals generation specially (ROS) resulted from excessive consumption of carbonated beverages , ROS cause reduction of spermatocytes and destroyed of epithelial tubular led to decrease in sperm count , D' Apolito *et al.* (2010) noted pro oxidation after chronic light cola drinking . Sajal *et al.* (2008) showed disorders in spermatogenesis by ROS which induce apoptosis in germ cells led to reduction of sperm .

Significant increasing in urea level and non-significant increasing in creatinine concentration were found in soft drinks exposed groups , this due to effect of soft beverage on kidney , naturally any disturbance in structure affected on function of kidney later causing accumulation of nitrogenous wastes as urea and creatinine . Akande and Banjoko (2011) revealed to disorders in kidney function in rats by treated with energy beverage and increasing of urea concentration , and this result agree with Otero-Losada *et al.* (2013) who reported high urea after cola drinking .

The result of the present study found a significant decreasing in liver enzymes (ALT,AST and ALP) which belong to effect of caffeine that present in carbonated beverages , Abara *et al.* (2007) reported that caffeine has role in reduction activity of liver enzymes , This agree with Chen *et al.* (2006) noted many side effects in hepatocytes resulted from caffeine . Significant increasing in glucose level of treated groups , this due to a large amount of carbohydrates in carbonated beverages which will be metabolize into glucose , Morengal *et al.* (2012) found content carbohydrates in soft drinks and he refers to consumption of carbonated

has also been associated with poor dietary habits , weight gain and type 2 diabetes, predominately in adults . This agree with Otero-Losada *et al.* (2013) and Palmer *et al.* (2008) who noted consumption of soft drinks linked with diabetes mellitus .

One of causes decreasing of albumin and total proteins was effect of soft drinks on liver which has important role in synthesis of proteins , histological sections of liver in present study clearly included destroyed of hepatocytes lead to disorders of liver function in protein synthesis . This disagree with Jumaah (2016) who observed high serum albumin after artificially sweetened drinks (as coca cola) consumption . Perhaps , increasing in cholesterol and triglycerides due to high exposure of zinc in carbonated beverages , Al-Samurai (2015) noted the zinc was skip allowed limits in Pepsi cola .

The results of current study showed occur histological changes in testes , kidney and liver of treated groups , these back to carbonated and energy beverages drinking , Amato *et al.* (1997) reported 25 separate side effects associated with the consumption of carbonated soft drinks . Necrosis that observed in sections may be due to high consumption of Pepsi and Tiger beverages as toxin to the cells of organs , this agree with Farber *et al.* (1981) who showed necrosis result from extrinsic insult for cell as toxic and osmotic effects . Necrosis may be belong to effect the carbon dioxide of Pepsi and Tiger on membranes of mitochondria lead to change in ATP production which reduce blood amount arrived of cells (hypoxia) consequently cells necrotic , This agreement with Al-Samurai (2015) who revealed that carbonated beverages consist of carbon dioxide more allowed limits .

Congestion back to effect of Pepsi and Tiger beverages in hypertension which causes expansion blood vessels and help to aggregation of blood cells that lead to congestion , Yoo *et al.* (2004) mentioned the consumption of sugar-sweetened soft drinks is also associated with many diseases including elevated blood pressure . While hemorrhage belong to rupture blood vessels by increasing blood pressure subsequently blood flow outside of their . Yoo *et al.* (2004) explained high blood pressure by soft drinks consumption . Glomeruli changes such as atrophy , absence ,

death of glomeruli and enlargement of Bowman's space may be caused by the effect of free radicals that are generated from long-term carbonated beverages in membranes of cells, Alisi *et al.* (2011) showed free radicals oxidative lipids of cell membranes.

Also, other histological damages regarding the intake of carbonated drinks, Fung *et al.* (2009) reported that consumption of carbonated beverages has been linked with development of structural and functional disorders of organs resulting in health problems. This result agrees with Adjene *et al.* (2010) and Passman *et al.* (2009) who revealed some congestion, diffuse glomerulonephritis, tubular necrosis, distortion and disruption of cytoarchitecture of renal cortex in kidney of rats by chronic consumption of soda pop drinks.

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دراسة التغيرات الفسلجية والنسجية في ذكور الجرذان المختبرية *Rattus norvegicus* بعد تناول المشروبات الغازية ومشروبات الطاقة

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الخلاصة

أجريت الدراسة الحالية لمعرفة تأثير تناول أحد أنواع المشروبات الغازية (الببسي كولا) ومشروبات الطاقة (التاىكر) في بعض المعايير الفسلجية والتغيرات النسجية المرضية لذكور الجرذان المختبرية *Rattus norvegicus* ، إذ أستعمل إثنان وثلاثون من ذكور الجرذان التي قسمت إلى أربع مجاميع جرعت المجموعة الأولى بالمحلول الفسلجي الملحي وعدت كمجموعة سيطرة وجرعت المجموعة الثانية بـ 1 مل/حيوان/يوم من الببسي كولا فيما جرعت المجموعة الثالثة بجرعتين من الببسي كولا بمقدار 1 مل/حيوان/يوم في كل جرعة وبفارق أربع ساعات بين الجرعتين ، بينما المجموعة الرابعة فقد جرعت بـ 1 مل/حيوان/يوم من مشروب الطاقة نوع Tiger . أوضحت نتائج الدراسة الحالية حصول إرتفاع معنوي ($P \leq 0.05$) في معدل وزن الجسم ونسبة تشوهات النطف واليوريا ومستوى سكر الدم والكليسيريدات الثلاثية في مجاميع الحيوانات المعاملة بالببسي والتاىكر مقارنة مع مجموعة السيطرة ، كما أظهرت النتائج إنخفاضاً معنوياً ($P \leq 0.05$) في العدد الكلي للنطف وإنزيمات الكبد (ALP وAST وALT) ومستوى الألبومين والبروتين الكلي للمجاميع المعاملة بالمشروبات الغازية (المجموعة الثانية والثالثة والرابعة) مقارنة مع مجموعة السيطرة . فيما لوحظ إرتفاعاً غير معنوياً في تركيز الكرياتنين والكولسترول للمجاميع المعاملة (بجرعة واحدة وجرعتين من الببسي وبمشروب الطاقة) مقارنة بمجموعة السيطرة . كما أظهرت نتائج الدراسة الحالية إن معاملة ذكور الجرذان المختبرية بالببسي والتاىكر سببت تغيرات نسجية في الخصى تمثلت بتنخر الطلائية وتحلل النسيج البيني وزيادة سمكه وإحتقان دموي وإنخفاض أعداد الخلايا المولدة للنطف ، كما تضمنت حصول الإحتقان وإرتشاح الخلايا الإلتهابية وضمور وفقدان الكبيبات الكلوية وموتها وتوسع حيز محفظة بومان إضافة إلى ظهور النزف الدموي بين النبيبات الكلوية ، بينما شملت التغيرات النسجية لأكباد الحيوانات المعاملة حدوث الإلتهاب والإحتقان وتوسع الجيبانيات وتضخم أنوية الخلايا الكبدية مع ظهور نزف دموي .

الكلمات المفتاحية: المشروبات الغازية ، النطف ، التغيرات النسجية .