

Original article

## Study The Effect of Some Energy Drinks on The Liver and Pancreas Functions in Male Albino Mice

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### Abstract

The current study aimed to determine the extent of the effects of energy drinks on the liver and pancreatic functions. The study was conducted on (35) male albino mice, whose weighting between (26-28 gm). The mice were divided into five groups (7/ group): the 1<sup>st</sup> group, received a diet and pure water, the 2<sup>nd</sup> group: which received an oral dose of 7.5mg/kg RB, the 3<sup>rd</sup> group received an oral dose of 15mg/kg RB, and the 4<sup>th</sup> group received an oral dose of 7.5 mg/kg PH and the 5<sup>th</sup> group were orally dosed with 15mg/kg PH, and after end of the experiment period, the mice were sacrificed and blood samples were collected for biochemical tests, liver and pancreas were extracted for histological study. The results obtained from the biochemical analysis showed that no significant differences were recorded in liver enzymes and liver tissue. While there was a significant increase  $p<0.05$  in glucose level in the 2<sup>nd</sup> group treated with 7mg/kg RB, and in cholesterol and HDL levels in the 4<sup>th</sup> and 5<sup>th</sup> groups that treated with 7.5mg/kg & 15mg/kg PH, as will as there was a significant increase  $p<0.05$  in the insulin hormone concentration in the 3<sup>rd</sup> group that treated with 15mg/kg RB. The results of the tissue samples showed that no changes were recorded in the liver tissues and cells, while there were cells necrosis, inflammation, fibrosis of pancreatic tissue, bacterial infection, and bacterial pancreatitis. These results indicate that energy drinks cause tissue damage.

**Keywords.** Energy Drinks, Liver, Pancreas, Mice.

### Introduction

Energy drinks are theoretically defined as: drinks that aim to accelerate the recovery process by providing metabolic energy (Carbohydrates), mental energy (Caffeine), and some other elements (Amino acids and Vitamins). While the operational definition is: liquids containing nutrients of varying proportions, mostly of an energy nature due to the metabolic and mental components, and other elements they contain. (Samir, 2015).

The term energy drinks also refer to any product in the form of a drink or concentrated liquid, which claims to contain a mixture of ingredients that have the property of raising the level of energy and vitality, and also contains high concentrations of Taurine, caffeine, and Glucuronolactone. These high concentrations can be responsible for harmful effects on health (Bath *et al.* 2012).

Energy drinks are also defined as drinks enhanced with added nutritional supplements and differ from soft drinks or sports drinks in terms of caffeine, as well as sugars and other nutritional supplements. Energy drinks are considered one of the most popular food products among athletes and ordinary people, due to the intensive advertising and huge budgets spent on them by the producing companies, with the aim of promoting their importance and highlighting their role in providing energy and delaying the feeling of physical and mental fatigue. Energy drinks also aim to provide the body with a large amount of metabolic energy and mental capacity in addition to amino acids and vitamins. The popularity of energy drinks and other energy products has spread in the past several years; however, their use is not without risks (Wolk *et al.* 2012).

It is advertised as increasing energy, improving athletic performance, concentration, reaction time, alertness, attention, emotions, metabolism, and reducing physical and mental stress. In 1949, energy drinks were introduced in the United States and became commercially available in Thailand. Modeled after Krating Daeng, they were marketed in Austria in 1987. Red Bull then became more popular in Europe and Asia in the 1990s. It is available in 140 countries worldwide, and more than 500 energy drinks were launched worldwide in 2006 (De Sanctis *et al.* 2017).

In 2007, the US Institute of Medicine published a report recommending a food environment for children and adolescents. Among the recommendations was to restrict carbonated, fortified, or flavored water. In 2011, increased consumption of Red Bull was reported in many countries around the world.

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The Research problem came from many directions, the most important of which is the consumption of energy drinks by students and adolescents, in addition to the large number of previous studies and their modernity on the consumption of energy drinks by individuals in universities, centers and sports cities, and the lack of research interest in Arab countries on the effect of energy drinks on individuals (Al-Mutafawi *et al.*, 2017).

Companies also relied on their marketing advertisements on the fact that these products are sports products that increase concentration and reduce the feeling of fatigue by supplying the body with energy. Therefore, the problem of this study was to determine the effect of this energy on some physiological changes. This study aims to investigate the effects of low and high doses of some energy drinks (Red Bull and Boom Boom) on the kidney and testis physiological functions of male albino mice.

## Materials and Methods

### Energy Drinks

In the current study, two types of energy drinks were chosen that are most popular in the Libyan markets, which are Red Bull and Power Hours.

### Experimental animals

Thirty-five adult, male *Mus musculus* mice (mean weight 26-28g) were kept in the Animal House of College Science, Elmergib University. The mice were acclimatized under a 12h/12h light/dark photoperiod and under normal, healthy laboratory conditions at a mean temperature of  $25 \pm 2$  °C. The experiments were carried out in line with the recommendations of the International Laboratory Animal Use and Care.

### Experimental Design

The animals were divided into five groups, 7 animals /each group. The first group was a control group, and was left without dosing and was served with distilled water daily orally for 30 days. The Animals of the 2<sup>nd</sup> group was given a low dose of (7.5 mg/kg) of Red Bull energy drink. The animals of 3<sup>th</sup> group was dosed orally with (15 mg/kg) of Red Bull energy drink. The 4<sup>th</sup> group was dosed orally with (7.5 mg/kg) of Power Hors energy drink. The five group was orally dosed with (15 mg/kg) of Power Hors energy drink. All treatments were given by gavage and lasted for 30 consecutive days.

### Handling And Sample Preparation

After (30) days of dosing, the mice were sacrificed after being fasted overnight. Blood samples were collected from the white mice using centrifuge tubes for determination of blood parameters. The samples were allowed to coagulate, and the sera were stored at -20 °C before assay. AST, ALT, and ALP activities are measured in ready-made solutions prepared by Biomaghareb Company, using a Spectrophotometer (230-BS Mindray), Bergmeyer *et al.*, 1978. As well as the total Cholesterol, HDL-C, triglycerides, and blood glucose levels were estimated using ready-made solutions prepared by Biomaghareb and using the German-made 4040V5 Photometer spectrophotometer (Tietz *et al.*, 1995). The insulin hormone in the sample was estimated using determined by enzyme linked immune sorbent assay (ELISA) method. Liver and pancreas were removed immediately for histopathological study.

### Histological examination

The sample **tissues** were collected from the liver and pancreas, then put in a 10% formalin solution until the experiment was performed on it, and it was extracted from the formalin. A series of operations was performed on it based on the described method (Schreibman Presnell and 1997).

### Statistical analysis

All data of the present study were analyzed statistically by using the Statistical Package for Social Sciences (SPSS) version 20. The values are expressed as a Mean  $\pm$  Standard error (M $\pm$ SE). One-way analysis of variance (ANOVA) was used to test for differences between treatment groups. The results were considered significant at ( $p < 0.05$ ). The least significant difference (LSD) was used to test the significance of the difference between groups of mice. Duncan's test (Homogeneity) was run to estimate the homogeneity in the studied parameters between all different groups of mice; values with significant variations were detected by superscript letters at  $p < 0.05$ .

## Results

### Body weight

The body weight (B.WT.gm) gain before and after administration of energy drinks was measured and compared. The results obtained from the (ANOVA) analysis showed that before starting the experiment, the mean body weight was approximately similar between groups. At the end of the experiment, the effect of energy drinks on the body weights was assessed, and the results are presented in Table 1. The results indicated that there was a significant change in the body weight before and after the experiment, with noticeable weight increases in some groups, suggesting that the experiment had a significant impact on the body weight. A significant increase ( $P < 0.05$ ) was observed in the animals treated with RB 15 mg/kg and PH15mg/kg compared to the group before the experiment, while there was a significant decrease ( $P < 0.05$ ) in the weights of the rats treated with RB 7 mg/kg and PH 7.5mg/kg after the experiment compared to their weights before the experiment.

**Table 1. The effect of daily administration of energy drinks (PH7.5mg/kg, PH 15mg/kg, and RB7.5mg/kg, RB 15 mg/kg) on the body weight (gm) of male albino mice after four weeks**

Groups	Control	RB7.5mg/kg	RB15mg/kg	PH7.5mg/kg	PH15mg/kg
Initial weight(gm)	22.86 $\pm$ 0.88 <sup>a</sup>	24.71 $\pm$ 1.04 <sup>ab</sup>	23.14 $\pm$ 0.86 <sup>a</sup>	28.42 $\pm$ 0.53 <sup>c</sup>	24.14 $\pm$ 1.42 <sup>ab</sup>
Final Weight(gm)	25.86 $\pm$ 0.94 <sup>bc</sup>	23.00 $\pm$ 0.53 <sup>a</sup>	28.14 $\pm$ 0.88 <sup>c</sup>	22.00 $\pm$ 0.65 <sup>a</sup>	27.86 $\pm$ 0.40 <sup>c</sup>
% Change	13.12*	-6.92	21.61	-22.59	15.41*

Values were expressed as mean $\pm$  SE for 7mice each group. The different letters in the table are significantly different at ( $p < 0.05$ ) when comparing between groups. % change = percentage of change in the body weight between the beginning and end of the experiment. (\*) = significant difference of less than  $p < (0.05)$ .

### Measurement of liver functions

Based on the data presented in (Table 2), the means AST, ALT, and ALP values showed no important differences ( $p > 0.05$ ) between the treated groups with energy drinks at concentrations of (PH7.5mg/kg, PH 15mg/kg, and RB7.5mg/kg, RB 15 mg/kg) compared to the control group.

**Table (2). Effect of daily administration of energy drinks (PH7.5mg/kg, PH 15mg/kg, and RB7.5mg/kg, RB 15 mg/kg) on the activities of serum Aspartate aminotransferase (AST) (u/l), Alkaline aminotransferase (ALT), and Alkaline phosphatase (ALP) of male albino mice after 4 weeks of the treatment**

Parameters		CONTROL	RB7.5mg/kg	RB15mg/kg	PH7.5mg/kg	PH15mg/kg
AST (U/L)	Mean $\pm$ SE	124.75 $\pm$ 16.11 <sup>a</sup>	122.50 $\pm$ 5.38 <sup>a</sup>	122.50 $\pm$ 7.24 <sup>a</sup>	124.75 $\pm$ 6.39 <sup>a</sup>	124.75 $\pm$ 2.75 <sup>a</sup>
	% of change from control		1.80	1.80	0.00	0.00
ALT (U/L)	Mean $\pm$ SE	42.75 $\pm$ 9.74 <sup>a</sup>	33.50 $\pm$ 0.28 <sup>a</sup>	41.50 $\pm$ 3.18 <sup>a</sup>	36.00 $\pm$ 3.24 <sup>a</sup>	36.25 $\pm$ 4.21 <sup>a</sup>
	% of change from control		-21.64	-2.92	-15.79	-15.20
ALP (U/L)	Mean $\pm$ SE	192.75 $\pm$ 12.31 <sup>a</sup>	195.75 $\pm$ 12.87 <sup>a</sup>	193.75 $\pm$ 3.54 <sup>a</sup>	154.50 $\pm$ 33.69 <sup>a</sup>	168.50 $\pm$ 10.76 <sup>a</sup>
	% of change from control		1.56	0.52	-19.84	-12.58

Values are given as mean  $\pm$  SE for 7 mice in each group. \*significant ( $p < 0.05$ ) as compared with the control group. The same letters in the row are not significantly different. (%) are calculated in comparison to the corresponding control group.

### Measurement of Lipid Profile

Data recorded in (Table 3) showed the effect of energy drinks administration in male white mice for 30 days on the four groups of different concentrations compared to the control group.

**Table (3). Effect of oral administration of energy drinks (PH7.5mg/kg, PH 15mg/kg, and RB7.5mg/kg, RB 15 mg/kg) on serum tri-glycerides, cholesterol, and High-density lipoprotein (HDL-C) (mg/dl) after 4 weeks of treatment in mice**

Parameters		Control	RB7.5mg/kg	RB 15mg/kg	PH7.5mg/kg	PH15mg/kg
Triglyceride s gm /dl	Mean± SE	85.33±17.21a	115.86±19.38ab	140.43±15.07b	133.14±9.41 b	79.14±14.62a
	%of change from control		35.78	64.57*	56.02*	-7.25
Total Cholesterol mg\dl	Mean±SE	106.25±11.20 a	163.75±10.42 a	167.50±8.77 a	149.00±19.40b	187.50±16.72 b
	%of change from control		54.12*	57.65*	40.24*	76.47*
HDL-CL mg\dl	Mean±SE	85.37±12.4 a	117.7±22.5 ab	111.25±24.1 a	171.25 ±20.16 b	131.25±4.26ab
	%of change from control		37.91	30.30	100.57*	53.72

Values are given as mean ± SE for 7 mice in each group. \* significant ( $p<0.05$ ) as compared with the control group.

The same letters in the row are not significantly different. (%) are calculated in comparison to the corresponding control group.

### Effect of energy drinks on serum triglyceride concentration

The data represented in (Table 3) showed that there is a significant ( $P<0.05$ ) increase observed in the 3rd and 4<sup>th</sup> groups (RB15 mg/kg and PH7.5mg/kg), with means of 140.43 and 133.14, with percentage changes of 64.57% and 56.02, respectively.

### Effect of energy drinks administration on serum total cholesterol concentration of white male mice

Effect of daily administration of energy drinks (7.5 mg\kg-15 mg\kg RB and 7.5mg\kg-15mg\kg PH) on serum Total Cholesterol level of male albino mice after four weeks of treatment is shown in (Table 3). The obtained results revealed a general significant increase ( $P<0.05$ ) in serum total cholesterol concentration after four weeks of administration of energy drinks (7.5 mg\kg-15 mg\kg RB and 7.5mg\kg-15mg\kg PH), values were 163.75, 167.50 ,149.00 and 187.50 mg\dl respectively when compared to control group which was 106.2 mg\dl, with percentage changes of 54.19%,57.73%,40.31% and 76.55% respectively.

### Effect of energy drinks on serum high-density cholesterol concentration

The effect of energy drinks on the high-density cholesterol shown in (Table 3), which dealt with the increase between the averages of This indicates that there is significant difference between the averages ( $p<0.05$ ), where the means of the two groups (RB7.5 mg\kg with a mean 117.75- PH7.5mg\kg with a mean of 171.25and group PH 15mg\kg with a mean of 131.25mg\dl, with percentage changes of 37.91%, 100.57% and 53.72% respectively.

### Measurements of glucose and insulin hormone in the blood of the target groups of the study

The Effect of oral administration of energy drinks (PH7.5mg/kg, PH 15mg/kg, and RB7.5mg/kg, RB 15 mg/kg) on serum glucose and insulin hormone levels after 4 weeks of treatment in male albino mice is shown in (Table 4).

**Table (4). Effect of oral administration of energy drinks (PH7.5mg/kg, PH 15mg/kg, and RB7.5mg/kg, RB 15 mg/kg) on serum glucose and insulin hormone levels after 4 weeks of treatment in male albino mice**

Parameters		Control	RB7.5mg/kg	RB15mg/kg	PH7.5mg/kg	PH15mg/kg
Glucose gm/dl	Mean±SE	107.67± 6.05 a	182.00± 20.27b	123.75±4.64 a	98.00± 10.88a	107.75 ± 7.90 a
	%of change from control		69.04*	14.93	-8.98	0.07
Insulin Hormone µu\ml	Mean±SE	8.95±0.64 a	20.53±4.78 b	50.00.±0.00 b	32.82 ±6.57 b	31.93±3.15 b
	%of change from control		129.39*	458.66*	266.70*	256.76*

Values are given as mean ± SE for 7 mice in each group. \* significant ( $p<0.05$ ) as compared with the control group. The same letters in the row are not significantly difference. (%) are calculated in comparison to the corresponding control group.



### Effect of energy drinks on glucose concentration

Table 4 shows the effect of daily administration of energy drinks (7.5 mg\kg-15 mg\kg RB and 7.5mg\kg-15mg\kg PH) on serum glucose concentration (mg\dl) of male albino mice after four weeks of treatment. As shown in the means of the groups, there was a significant increase at  $P<0.05$  in the mean of the second group (RB7.5 mg/dl) with a value of 182.00 and a percentage of 69.04% when compared to the control group with a mean of 107.67.

### Effect of energy drinks on Insulin Hormone concentration

Effect of daily administration of energy drinks (7.5 mg\kg-15 mg\kg RB and 7.5mg\kg-15mg\kg PH) on serum Insulin Hormone level ( $\mu\text{u}\backslash\text{ml}$ ) of male albino mice after four weeks of treatment as shown in (Table 4). The obtained results revealed a general significant increase( $P<0.05$ ) effect on serum Insulin Hormone level, values were 20.52,50.00,32.82 and 31.93  $\mu\text{u}\backslash\text{ml}$  respectively when compared to the control group, which was 8.95  $\mu\text{u}\backslash\text{ml}$ , with percentage changes of 129.39%,458.66%,266.70% and 256.76% respectively.

### Histopathological study:

#### Effect of Energy Drinks on the Liver

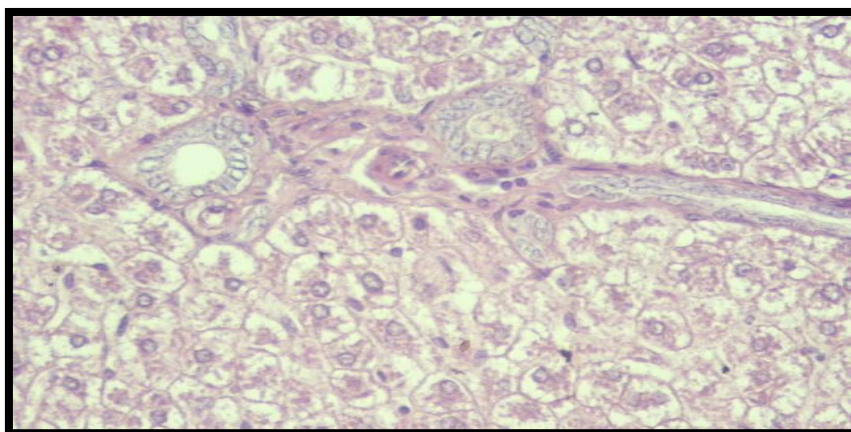


Figure (5). showing cross –sectional view of the liver of white mic stained with hematoxylin and eosin (400 X-E&H). Shows the normal tissue structure in the control group. CV= Central vein, h= hepatocytes, PV= Portal vein

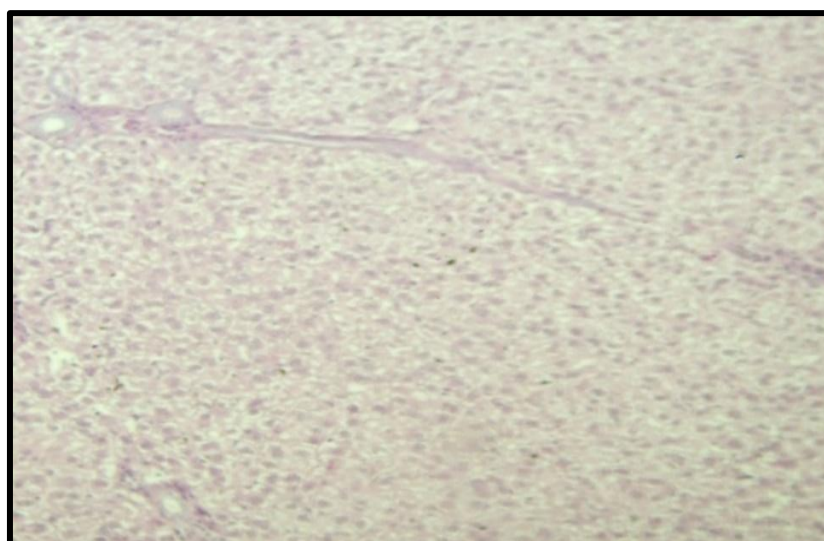


Figure (6). showing cross –sectional view of the liver of white mic stained with hematoxylin and eosin (400 X-E&H). A section of the liver from the group treated with (RB7.5 mg/kg) showed a high cell density; the dark purple color indicates high cellular activity.

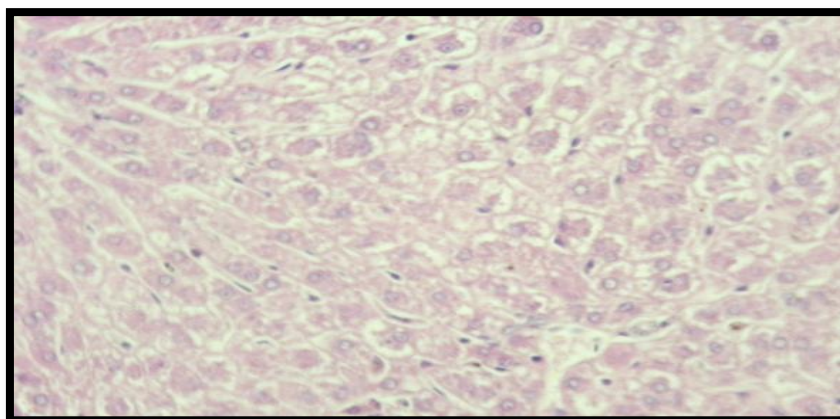


Figure (7). showing cross –sectional view of the liver of white mic stained with hematoxylin and eosin (40 X-E&H). A section of the liver from the group treated with RB15 mg\kg showed normal tissue structure; it appears to have an uneven distribution of cells

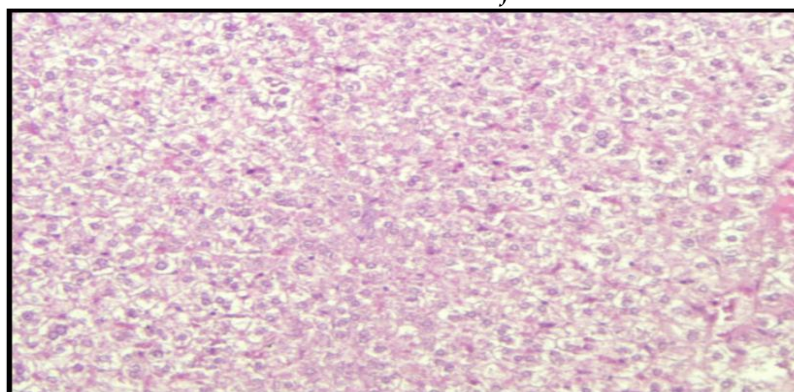


Figure (8). showing cross –sectional view of the liver of white mic stained with hematoxylin and eosin (40 X-E&H). A section of the liver from the group treated with PH7.5 mg/kg, showed a Hepatocytes, regular in shape, sinusoids, and normal distribution, reflecting proper blood flow in the tissue

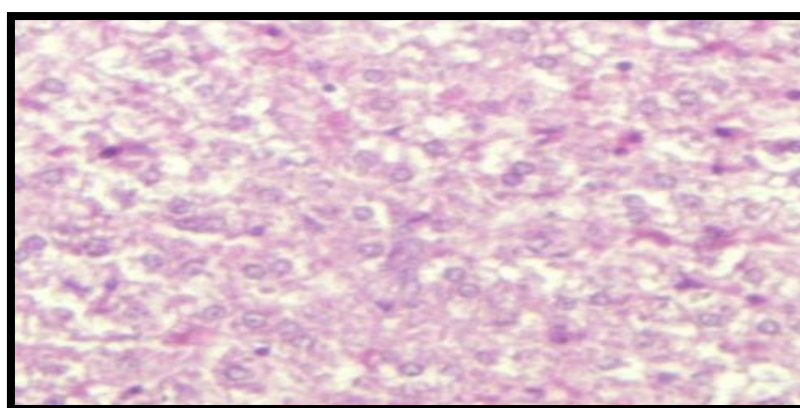


Figure (9). showing cross –sectional view of the liver of white mic stained with hematoxylin and eosin (40 X-E&H). A section of the liver from the group treated with PH15 mg/kg, showing no signs of fibrosis, inflammation, or increased connective tissue

### Effect of Energy Drinks on the Pancreas



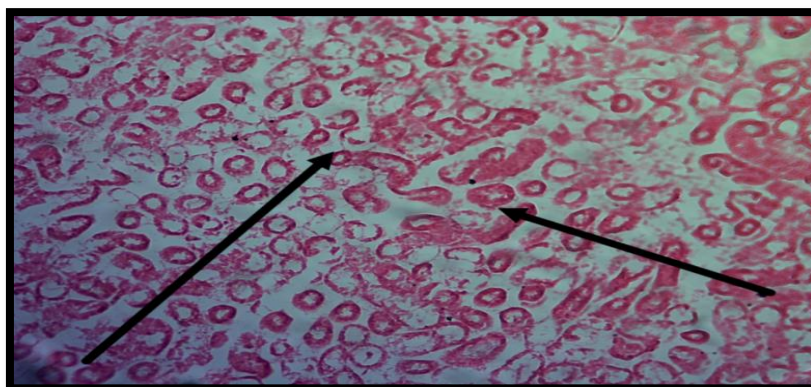


Figure (10). showing cross –sectional view of the Pancreas of white mic stained with hematoxylin and eosin (40 X-E&H). The arrows point to Pancreatic acini, the dark-stained cells reflect their secretory nature, and supporting connective tissue can be seen separating and surrounding the acini



Figure (11). showing cross –sectional view of the Pancreas of white mic stained with hematoxylin and eosin (40 X-E&H). A section of the Pancreas from the group treated with (RB7.5 mg/kg), the Arrow (A) represents necrotic tissue or damaged remnants of pancreatic tissue, (B) represents bacterial infection (bacterial clusters)

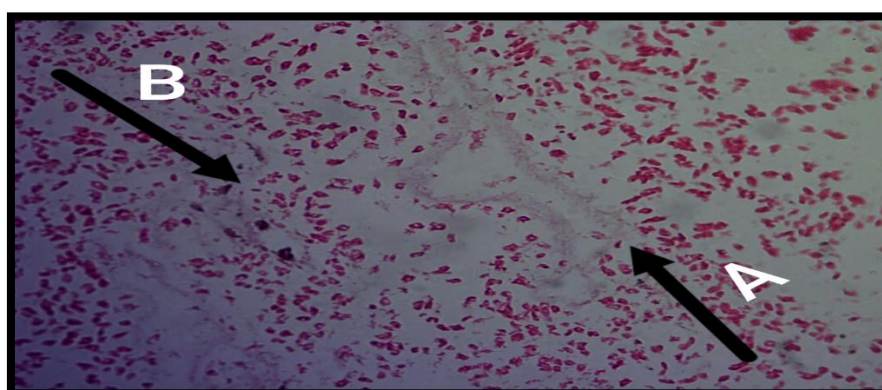
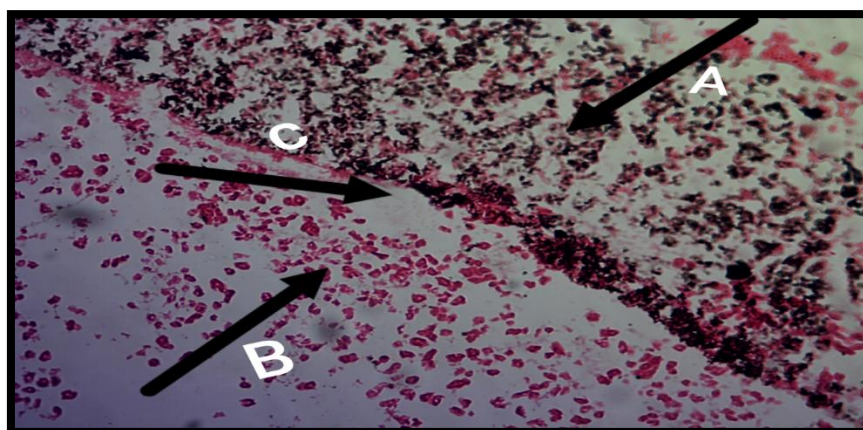
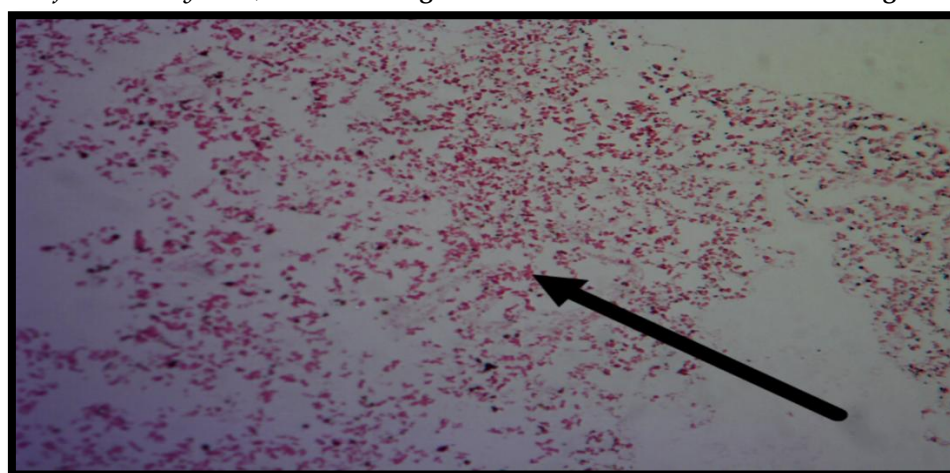


Figure (12). showing cross –sectional view of the Pancreas of white mic stained with hematoxylin and eosin (40 X-E&H). A section of the Pancreas from the group treated with (RB15 mg/kg), the Arrow (A) points to a non-cellular structure that appears wavy or fibrous and necrosis and fibrotic deposits, (B) indicates bacterial clusters or small circular



**Figure (13).** showing cross –sectional view of the Pancreas of white mic stained with hematoxylin and eosin (40 X- E&H) A section of the Pancreas from the group treated with( PH7.5 mg\kg), The Arrows, (A) shows a dense aggregation of dark, irregularly shaped cells, (B)shows more distinguishable cells with prominent nuclei, and inflammatory cells, (C) a dividing line is visible between two distinct regions



**Figure (14).** showing cross –sectional view of the Pancreas of white mic stained with hematoxylin and eosin (40 X- E&H). A section of the Pancreas from the group treated with (PH15 mg/kg), the arrow points to small clusters of bacteria with a rod or cocci shape, appearing pink. red, the presence of these bacteria in a pancreatic sample may suggest a bacterial infection

## Discussion

Energy drinks refer to dietary food products in which the main source of energy is carbohydrates. The main active constituents of energy drinks include varying amounts of caffeine, guarana extract, taurine, and ginseng. Also, additional amino acids, vitamins, and carbohydrates are usually present. The intended effects of energy drinks are to provide sustenance and improve performance, concentration, and endurance. (Sadowska, 2012). The amounts of guarana, taurine, and ginseng found in popular energy drinks are far below the amounts expected to cause either therapeutic benefits or adverse events. However, caffeine and sugar are present in amounts known to cause a variety of adverse health effects (Clauson *et al* 2008). Effect of EDs on body weight changes in different mouse groups. A significant change in the weights after the experiment. The results of our study showed a significant decrease in the body weights of rats treated with the energy drinks (RB7.5 mg/kg and PH 7.5 mg/kg) compared to the control group. This reduction may be attributed to the physiological effects of caffeine and taurine, which are known to increase basal metabolic rate and stimulate the central nervous system, leading to higher energy expenditure and appetite suppression. Our findings are consistent with those of (Aldreeny.2018), who reported weight loss in rats administered energy drinks and linked it to the enhanced metabolic activity caused by their components. Similarly, (Sherif. 2019) highlighted the role of caffeine and taurine in reducing appetite and increasing physical activity, which contributed to weight reduction. In the same



context, (Al-Majed. *et al.*, 2018) found that energy drinks led to weight loss by elevating metabolism and increasing caloric consumption. There was a noticeable increase in body weight in both the (RB 15 mg/kg and PH 15 mg/kg) groups, which can be attributed to the sugar content of energy drinks. For example, Red Bull contains approximately 27 grams of sugar per can, which increases the total daily caloric intake of the rats, leading to fat storage and weight gain. These findings are consistent with the results of (Abdel-Kareem. *et al.*, 2023), who reported that the consumption of energy drinks led to a significant increase in body weight, along with elevated glucose and insulin levels — indicating the development of insulin resistance, a key factor associated with weight gain and fat accumulation. Similarly, the study by (Akande and Banjoko, 2011) supported these results, showing that rats treated with Power Horse for 28 days exhibited elevated liver enzymes (ALT, AST, and ALP), along with histological changes in the liver and kidneys. These findings suggest impaired metabolism and potential long-term toxicity, which may contribute to increased body weight. Evaluating the functions of the liver and pancreas is very important in evaluating the extent of the effect of energy drinks. AST, ALT, and ALP activities are usually measured to monitor liver damage. Mild or high activity in AST indicates liver injury.

The results of the current study showed that non-significant deference of the levels of AST, ALT, and ALP enzymes, This may be attributed to several factors. The short duration of the experiment may require longer periods to manifest. The dose used was lower than the toxic dose. The administered dose might not have reached the threshold needed to cause significant changes in liver enzymes and liver defense mechanisms. The liver can detoxify certain harmful substances to a certain extent before signs of damage appear. Aloe's uneven distribution of toxic components, caffeine, and taurine may not affect the liver as strongly as they affect other organs, and differences in animal species or genetic makeup: Some strains of rats are more resistant to the effects of certain substances. These results are in agreement with the study by (Al-Otaibi, 2018), which did not report significant changes in liver enzymes following the consumption of energy drinks in rats, and also with the study by (Al-Siddiqi, 2016), which indicated that hepatic effects may only appear with higher doses or prolonged exposure. This Studies Consistent with Our Results (Al-Majed *et al.*, 2018), found no significant changes in AST and ALT levels after energy drink consumption, which aligns with our results showing stable enzyme levels. (Ahmed. *et al.*, 2020) indicated that consuming energy drinks at low and moderate doses did not cause significant changes in liver function, which is consistent with our findings of no noticeable differences. (Khan, *et al.*, 2019) confirmed that energy drinks had no significant effect on liver enzymes, supporting our results that showed only minor, non-significant variations. A study by (Ibrahim and Iftikar. 2014), showed that RB intake has an effect on liver enzyme levels compared to the control group. While a study showed that consuming (PH) has harmful risks to the liver and its enzymes, as it increased the activities of ALP, ALT significantly decreased, while AST decreased (Akande and Banyoko, 2011). Another study conducted using three types of energy drinks (Red Bull-Power Hors -Code Red) reported that it caused an increase in liver enzymes ALT, ALP, and AST, and that the energy drink (PH) was more effective in its action on liver enzymes (RB) due to the difference in the mixture of its components (Khayyat *et al.* 2015). A study was conducted on rabbits using two types of energy drinks (PH, RB). A recent study showed a decrease in liver enzymes ALT, ALP, and AST. In contrast, a study showed that the components of energy drinks, both taurine and caffeine, lead to an increase in liver function (Chen *et al.*, 2019). Also, a graphic study revealed that is energy drinks beverages intake of high amount are hazardous on liver of albino rats (Shalaby. *et al.*, 2024). Also (Malika, 2022) reported a significant increase in AST, ALT, and ALP levels compared to the control group, suggesting a negative impact of energy drinks on liver function. This contrasts with our findings, which showed no major changes. Ismail (2023) reported that energy drinks significantly influenced liver enzyme activity but did not cause noticeable histological changes in liver tissue. This partially contradicts our results, which showed no significant enzymatic changes. When measuring cholesterol levels, there was a significant increase in all the second and third groups treated with (RB 7.5mg\kg and RB 15mg\kg) and the fourth and fifth groups treated with (PH7.5mg\kg and PH15mg\kg) were close to each other when compared to the control group, perhaps the reason is sugar and caffeine content in energy drinks these components elevate cortisol levels in the body, which affects lipid metabolism and may increase cholesterol production in the liver. Taurine compound has a dual role; some studies have found that it may reduce cholesterol levels, while others have shown it contributes to lipid imbalance when used in high doses or in combination with caffeine. Energy drinks may affect the levels of LDL (bad cholesterol) and HDL (good cholesterol), and can contribute to hyperlipidemia. Our findings are consistent with those reported by (Al-Harbi, 2019). Our research demonstrated a statistically significant correlation between red bull energy drink consumption and increased total cholesterol levels (Mansour, 2021). The study suggested

that prolonged consumption of energy drinks results in elevated cholesterol levels, likely attributed to alterations in lipid metabolism. A slight increase was observed in the serum level of HDL in all experimental groups were close to that of the control group. This effect may be attributed to the active components such as caffeine and taurine, which can stimulate metabolism and improve the ratio of good fats in the blood. These findings are consistent with studies by (Al-otaibi,2018), which reported that chronic consumption of energy drinks led to a slight increase in HDL levels in rats, attributed to the effects of caffeine and taurine, and (Al-Eisaei, 2020) found that moderate doses of energy drinks increased HDL without significantly affecting total cholesterol or LDL. The effect of energy drinks on the four groups on triglyceride levels was that there was a clear increase in the third group treated with (RB15 mg/kg) and the fourth group treated with (PH 7.5mg\kg) when compared to the control group, which may be attributed to the content of simple sugars and caffeine in these beverages, both of which promote hepatic lipid synthesis. These findings are consistent with those reported by (Emmanuel (2013 and Al-Otaibi (2018) Reported a noticeable increase in triglyceride levels after administration of energy drinks, and Al-Harbi (2019), who found elevated triglycerides and cholesterol after four weeks of energy drink consumption. There was a clear effect on glucose levels in the second group treated with (RB 7.5 mg/kg) compared to the control group. This effect is attributed to the content of these drinks, which includes caffeine, simple sugars, and taurine, all known to directly affect carbohydrate metabolism and insulin regulation. Caffeine is believed to increase the secretion of stress hormones such as adrenaline, which enhances hepatic glucose production and reduces cellular insulin sensitivity, while the high sugar content causes a sharp rise in blood glucose levels. Taurine may also play an additional role in modulating metabolic responses; these findings are consistent with those reported by (Amin and Nagy,2009), who demonstrated that energy drink consumption led to notable changes in glucose metabolism in rats. Similarly, (Alsunni and Badar, 2011) highlighted the negative effects of energy drink components on glucose levels and related hormones. Furthermore, the master's thesis by (Al-Sharif ,2021) supported these results by showing significant increases in blood glucose and impaired insulin response in rats exposed to energy drinks. In contrast, some studies do not align with our findings. The reduction in serum glucose in the fourth group treated with (PH7.5mg\kg) compared to the control group in our results is consistent with the study that there is a significant decrease in blood sugar (glucose) in the group treated with (RB) compared to the group treated with (PH), the increase in blood sugar glucose was clear when compared to (RB) and the control group (Ismail, 2023). While (Jadowski, 2012) explained that there is an increase in insulin resistance and decreased glucose uptake in skeletal muscle due to carbohydrate oxidation, due to increased plasma glucose levels. Also, the elevation of insulin hormone levels in this study in all groups agreed with the results of (Ismail 2023) that there is a clear increase in insulin levels in the groups treated with the Energy drinks (PH & RB) compared to the control group. Ayuop *et al.* (2016) showed that PH energy drink increases the stimulation of (Beta cells) in the pancreas and a significant increase in insulin and glucose levels, and taurine in the energy drink is associated with increased insulin secretion from the pancreas (Song, 2022). This is consistent with the study (Ragsdale2010), which showed a significant increase in blood sugar levels. Hanna *et al.* (2025) also mentioned that the high concentration of sugar, in addition to niacin, in the energy drink changes the metabolism of carbohydrates after consumption, which leads to an increase in blood glucose and insulin levels. The process of microscopic preparations, although very complex and precise, is of great importance in scientific and diagnostic research. It is considered an important tool in anatomical pathology and the first basis for diagnosing the disease due to its accuracy in diagnosis. Therefore, the objectives of this study were to determine the extent of the effect of energy drinks on the structure of the liver and pancreas. This study compares the histological changes that occur after treating white mice with energy drinks at different concentrations of RB PH, as observed in microscopic histological sections. Sections examined from the submitted hepatic tissue revealed normal hepatic lobules and hepatocytes with no evidence of significant pathological abnormality. This is consistent with the histological study that showed that energy drinks PH and RB do not significantly affect the anatomy of the liver (Ebuehi *et al.*, 2011). This is consistent with our results. As indicated by (Al-Khayat. 2015), there is liver toxicity in the PH and RB mouse groups, with the presence of necrotic areas and more obvious fascicular nuclei in the PH and RB groups. Concerning the liver tissue of rats given PH for 2 weeks, there were no obvious changes except widening between the hepatic strands and the appearance of mitotic division. Liver sections of animals given RB showed some signs of distortion in tissue after 2 weeks. Also (Hanna *et al.*, 2024), the consumption of energy drinks triggered histopathological changes in the liver (liver damage). The results of the experimental histological study conducted on the pancreatic sections stained with hematoxylin-eosin of mice treated with energy drinks showed normal observations in the control group. The pancreatic acini were observed as secretory

units composed of short cuboidal or columnar epithelial cells under a small lumen. The dark color of the cells reflects their secretory nature, as they are rich in enzymes and proteins. The connective tissues that support and separate the acini can also be seen. The islets of Langerhans may be present, but they are often lighter in color than the acini due to their endocrine nature. No cellular manifestations or changes indicating tissue damage were seen. As for the rest of the groups treated with energy drinks, they showed damage of varying levels, as the group treated with (7.5mg/kg RB), showed that there was a pathological process, as Arrow (A) indicates a non-background material with a pale appearance that may be tissue necrosis, which is a result of damage to the pancreatic cells, due to inflammation or infection, or remnants of a basement membrane or fibrous material resulting from the decomposition of normal tissue, due to severe inflammation or extracellular deposits that may be a sign of a chronic inflammatory response or a repair process. This area is evidence of damage to the pancreatic tissue, while Arrow (B) indicates clusters of small, dark, circular cells that may be bacteria. The presence of bacteria is associated with the process of suppurative pancreatitis or tissue necrosis. While the group treated with (15mg/kg RB) showed that the sample contains a mixture of mostly inflammatory cells or neutrophils and non-cellular structures, as well as the distribution of small cells indicates the possibility of inflammation or bacterial infection, and the background appears to be composed of interstitial material or damaged tissue, perhaps due to infection, as Arrow (A) indicates damaged tissue or cellular debris and Arrow (B) indicates the bacteria causing the infection. This is consistent with ((Hanna *et al.* (2025)), that the high concentration of sugar and jasmine in the energy drink leads to a change in carbohydrate metabolism, which leads to an increase in blood glucose and insulin levels, as well as an enlargement of the islets of Langerhans after consuming RB, and that increasing the dose and period of consuming the energy drink will lead to an increase in blood glucose levels and thus an increase in the production of reactive oxygen species, which leads to oxidative stress and damage to pancreatic tissue. As (Qasim. *et al.*, 2022) explained, the energy drink RB causes vascular congestion of the islets of Langerhans, as well as an increase in the size of the islets and necrosis of cells, as well as the decomposition of inflammatory mononuclear cells. The nucleus, as (Ismail. *et al.*, 2023) explained, RB energy drink may cause deformation of the pancreas and increase collagen fibers around blood vessels and ducts. While the group treated with (7.5 PBmg\kg), there are clear changes, as Arrow (A) indicates a dense cluster of dark-colored and irregularly shaped cells. These may be tolerant cells or necrotic dead tissue, which is an indicator of acute or chronic pancreatic injury, and may contain remnants of inflammatory materials such as white blood cells or fibrosis products. While Arrow B indicates scattered cells containing more prominent cells with prominent nuclei, there appears to be a spread of inflammatory cells or effects on normal cells, and Arrow C shows a dividing line between two different areas, the upper and lower areas. While the treatment group with (15mg/kg PH) indicates small clusters of rod-shaped or spherical bacteria that appear pink, which means Gram-positive bacteria, and the presence of these bacteria in the pancreas sample indicates a bacterial infection or bacterial pancreatitis, which occurs in cases of complicated inflammation or pancreatic abscesses. There is a heterogeneous background showing tissue debris or tolerant protein materials. The light areas and thin threads may represent tissue fibers or secretions. This is consistent with (Haroun *et al.*, 2020) that consuming an energy drink causes inflammation in the pancreas, due to the high caffeine content and the interaction between it and taurine as a component in energy drinks.

## Conclusion

The results of this study demonstrate that exposure of mice to two doses of Red bull and bower house (PH7.5mg/kg, PH 15mg/kg and RB7.5mg/kg, RB 15 mg/kg) for 4 weeks leads to occurrence slight change in the liver enzymes activities, pronounced elevation in the levels of triglycerides, cholesterol, glucose and insulin hormone in the serum suggests that the harmful effects of these EDs on metabolism proses and enzymes activities. these results call for restraint and caution in the consumption of Red Bull and Bower House and other EDs. Thus, the need for adequate public awareness cannot be overemphasized. The results of our study, obtained from the biochemical analysis and histopathological symptoms, showed that there was no direct effect on liver functions or liver tissue, while there was an effect on the pancreas, as there was an increase in glucose and insulin hormone. The presence of necrosis and inflammation in the pancreatic tissues indicates that energy drinks cause tissue damage, and therefore, caution should be exercised when consuming them due to their contents, including caffeine. Until more research reveals safe amounts of EDs and their ingredients, excessive use should be avoided, and a thorough history should include questions regarding supplements, including beverages such as EDs, which are perceived as safe.



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