



## ORIGINAL ARTICLE

# The Toxic Effect of Energy Drinks on The Structure of Pancreas of Adult Male Albino Rats.

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**Submit Date:** 23-04-2019

**Revise Date:** 22-05-2019

**Accept Date:** 28-05-2019

### ABSTRACT

**Background:** A controversy developed between the benefits of energy drinks versus the possible health threats since its revolution. Lack of information was a call to assess the effect of chronic consumption of Red Bull (RB) as one of the energy drinks, on the structure of the pancreas. The Aim of this Work is to evaluate the effect of Energy drinks (Red Bull) on rat pancreas and ability of rat pancreas to recover from effects of Red Bull. **Methods:** Thirty adult male albino rats divided into three groups, control, treated and recovered. Treated group was received 7.5 ml of RB by oral gavage daily for 4 weeks. Recovered group was left to recover for 15 days without any additional treatment. The rats will be anaesthetized and sacrificed. Pancreas prepared for light microscopic examination and tissue homogenate. **Results:** Red Bull (RB) induced pancreatic injury in the rats evidenced by histopathological alterations like, distorted pancreatic acini with vacuolated cytoplasm and vacuolated islets of Langerhans. Furthermore, RB administration caused oxidative damage by decreasing in reduced glutathione (GSH) and superoxide dismutase (SOD) levels in the pancreatic tissue. Stoppage of RB exposure resulted in amelioration in the histological alterations as compared with the RB treated rats. **Conclusion:** RB produces degenerative changes in the pancreas and causes oxidative stress. Stoppage of RB exposure results in some restoration of the normal histological structure of the pancreas. So, it is recommended to stop the usage of RB.

**Keywords:** Pancreas, Energy drinks, Red Bull, GSH, SOD.

### INTRODUCTION

Energy drinks are group of beverages that have gained their fame since 1997 [1]. They are designed to provide the consumer by a combination of stimulants and energy boosters that increase the physical endurance and concentration; improves cognitive as well as muscular performance, and provide mood enhancement [2, 3].

The main active ingredient in energy drinks is caffeine, and other substances such as taurine, carbohydrates, riboflavin, pyridoxine,

nicotinamide, vitamin B-complex, and various herbal derivatives such as ginseng are also present [4,5].

Caffeinated energy drinks is associated with several side effects such as insomnia, nervousness, restlessness, gastric irritation, nausea, vomiting, tachycardia, tremors and anxiety [6,7]. Moreover, it is associated with many cardiovascular health problems and diabetic complications [8,9].

Red Bull is the most popular energy drink consumed in Egypt [10]. The company

say that Red Bull “gives you wings” recommends that its consumption will improve the performance physically and mentally and also give the consumer a lot of energy. Its consumption is associated with a decrease in the heart rate and an increase in the systolic blood pressure and pain tolerance [10].

### METHODS

The study was performed according to The Institutional Animal Care and Use Committee Zagazig University (ZU-IACUC ) Instructions. The study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. Experiments complied with the ARRIVE guidelines and was carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animal.

**Chemicals:** Red Bull Can: was purchased from Egyptian markets.

Each 100 ml of Red Bull contains: taurine (400 mg), sucrose and glucose (11.3 g), B12 (0.4mcg), caffeine C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub> (32mg), gluconolactone (240 mg), niacin (7.2 mg), B6 (0.8 mg), panthenol (2.4 mg), inositol (20 mg), B2 (0.64 mg), artificial flavoring and sparkling water.

**Experimental animals and dosing:** This study was carried out on 30 adult male albino rats (five months ago) weighing (210-250 gm) for each. They were obtained from the Animal House of Faculty of Medicine, Zagazig University. All animals were kept under hygienic conditions. Standard food and tap water were administrated. They were housed in fan ventilated wide polypropylene cages with stainless steel tops and wood shavings for bedding. Temperature was maintained at 23±2°C. They were accommodated to the laboratory conditions for 15 days before being experimented. All the rats were handled in an accordance to the standard guide for the care and use of laboratory animals.

The rats were divided into 3 groups as follows:

**Group1 (control group)** was formed of 10 rats, which were given 7.5 ml normal saline by oral gavage daily for 4 weeks to measure the basic parameters.

**Group2 (Red Bull treated group)** was formed of 10 rats, which were given 7.5 ml of RB by oral gavage daily for 4weeks [11].

**Group3 (withdrawal group)** was formed of 10 rats, which were given 7.5 ml of RB by oral gavage daily for 4 weeks, and were left without treatment for another 15 days [11].

**Methods:** By the end of the experiment, all the animals were anesthetized by an intra-peritoneal injection of thiopental 30mg/kg body weight then sacrificed. Pancreatic specimens were taken for histopathological studies and biochemical studies. In addition, statistical analysis was performed.

**Biochemical studies:** Specimens were minced and homogenized (10% W/V) separately in an ice-cold saline, sucrose buffer (0.25 M sucrose, 1 mM EDTA and 0.05 M Tris-HCl, pH 7.4) in a Thomas Sci. Co. glass-type homogenizer (Teflon pestle). The homogenization of the tissues was carried out in Teflon-glass homogenizer with a buffer containing 1.15% KCl to obtain 1:10 (W/V) whole homogenate. Homogenates were centrifuged at 3000 r.p.m (+4 °C) for 15 min to determine superoxide dismutase (SOD) activity while, homogenates were centrifuged at 5000 r.p.m for 50 min to measure the reduced glutathione (GSH) activity [12].

**Histopathological studies:** Specimens were processed to be submitted to the light microscopic examination (Haematoxylin and Eosin (H&E)) [13].

**Statistical analysis:** There were highly statistical significant differences between the studied groups in SOD and GSH levels using ANOVA test as in table (1)

Using LSD to find a relation in-between the groups showed that the control group showed a highly statistical significant difference from RB group and a significant difference from withdrawal group in SOD and GSH levels. There were statistical significance differences between RB group and withdrawal group in SOD and GSH levels as in tables (2 & 3).

### RESULTS

**Light Microscopic Examination****Group 1 (Control group):**

H&E stained Sections of control group of an adult male rat pancreas showed thin connective tissue septa dividing the gland into well-developed pancreatic lobules of variable size and shape. These lobules were tightly packed and consisted of an exocrine part (acini & ducts) and an endocrine part (islets of Langerhans). The acinar tissue was predominant. Islets of Langerhans was embedded within the lobules and appeared as a pale stained area between the acini. (**Figure 1A**).

The exocrine portion of pancreas consisted of serous acini of typical form and appearance. They were well developed, tightly packed and appeared rounded or oval in shape. The pyramidal acinar cells were characterized by pale basal rounded nuclei, apical acidophilic cytoplasm packed with secretory granules and basal basophilic cytoplasm. The acinar boundaries were regular and well defined. Islets of Langerhans appeared pale in between the acini. Their cells were characterized by pale

nuclei. Blood capillaries were observed in between the islet cells. The ducts were seen lined by acuboidal epithelium (**Figure 1B**).

**Group 2 (RB treated group):**

H&E stained Sections of RB group of an adult male rat pancreas showed pancreatic lobules separated by dilated interlobular septa. Dilated ducts were seen (**Figure 2A**).

The pancreatic acini were distorted and characterized by the dark basal nuclei and the vacuolated cytoplasm (**Figure 2B**).

The islets of Langerhans showed intracytoplasmic vacuoles and Karyolysis (disappearance of nucleus) (**Figure 2C**).

**Group 3 (withdrawal group):**

H&E stained Sections of this group showed an improvement compared with RB treated group. The interlobular septa between pancreatic lobules were slightly dilated, dilated ducts were seen (**Figure 3A**). The pancreatic acini appeared slightly normal but some acinar cells vacuolated with dark nuclei. The islets of Langerhans exhibited some vacuolations (**Figures 3B,3C**). Dilated ducts lined by a flat epithelium were seen (**Figure 3C**).

**Table 1.** Comparisons between oxidative markers levels in the different studied groups using ANOVA (analysis of variance) test:

Group	Control (N=10)	RB (N=10)	withdrawal )N=10(	F	P
<b>Variable</b>					
SOD: (u/g)					
Mean ± SD	4.85 ± 0.02	3.24 ± 0.05	3.78 ± 0.04	623.4	<0.001
Range	4.83 – 4.86	3.17 – 3.29	3.75 – 3.85		**
GSH: (nmol/g)					
Mean ± SD	6.48 ± 0.16	3.90 ± 0.05	4.56 ± 0.15	189.3	<0.001
Range	6.28 – 6.66	3.85 – 3.96	4.42 – 4.77		**

SD: Standard deviation.

F: ANOVA test.

P:p-value

\*\* : highly significant (p<0.001).

N: Number of rats for each group.

**Table 2.** Least significant difference test (LSD) for comparison of SOD level in-between between the groups:

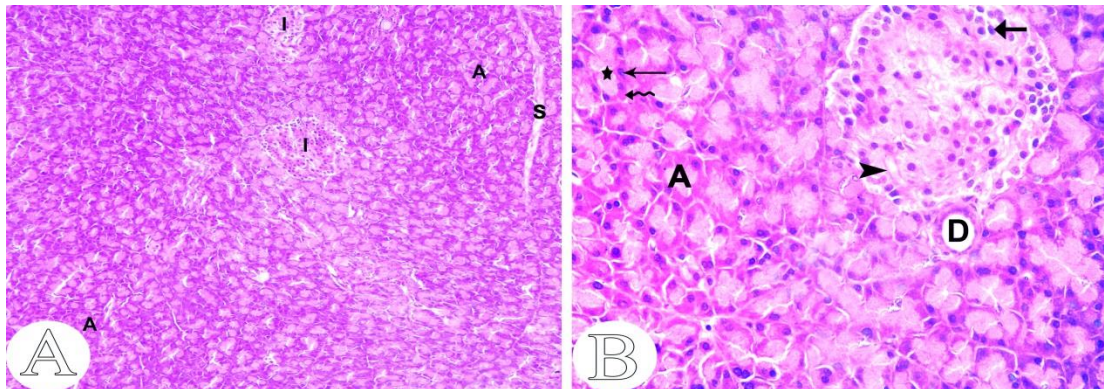
Group	RB (N=10)	withdrawal) N=10(
Control (N=10)	<0.001 **	<0.05 *
RB (N=10)		<0.05 *

\*: Significant. \*\*: Highly significant.  
N: Number of rats for each group.

**Table 3.** Least significant difference test (LSD) for comparison of GSH level in-between groups:

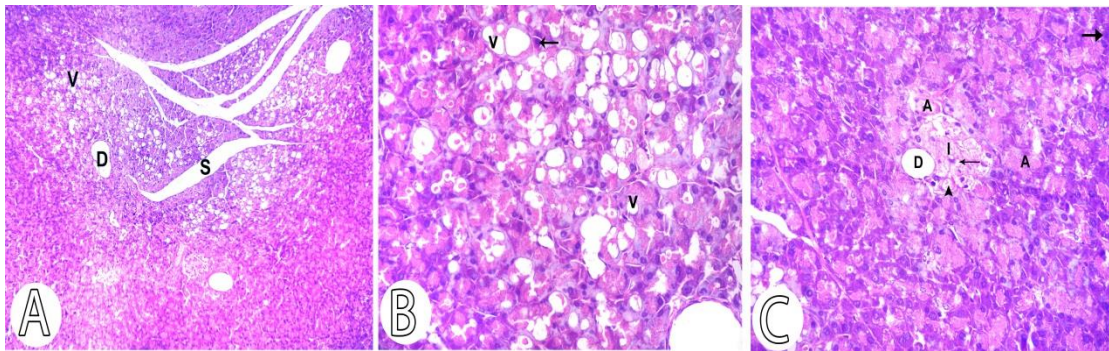
Group	RB (N=10)	withdrawal) N=10(
Control (N=10)	<0.001 **	<0.05 *
RB (N=10)		<0.05 *

\*: Significant. \*\*: Highly significant.  
N: Number of rats for each group.

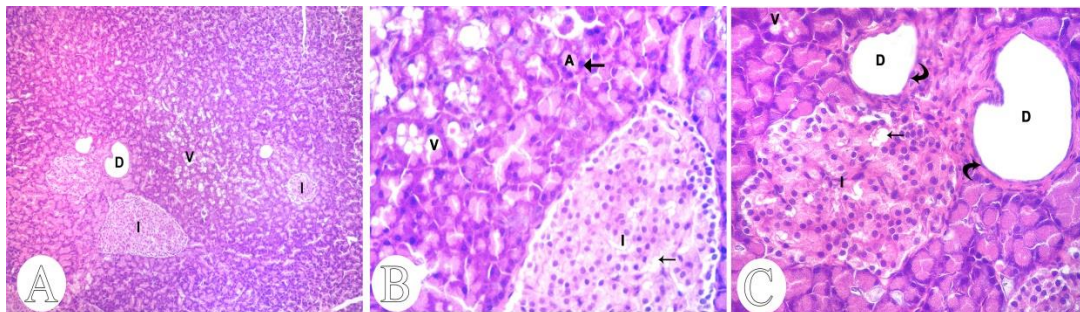


**Figure1.** A photomicrograph of a section of an adult male albino rat pancreas (control group) showing its general architecture; **1A:** showing serous acini (A), thin septa (S), and a pale stained islet (I). **1B:** showing serous acini (A) lined by pyramidal cells with basal rounded vesicular nuclei (thin arrow), apical acidophilic granular cytoplasm (\*) and basal basophilic cytoplasm (zigzag arrow). Duct appears between the acini (D). The cells of islet have pale nuclei (thick arrow). Blood capillaries are observed in between the islet cells (arrow head). **(H&E 1A x 100) (H&E 1B x 400).**





**Figure 2.** A photomicrograph of a section of an adult male albino rat pancreas (RB group) showing loss of its general architecture. **2A:** The pancreatic acini are irregular in shape with vacuolations (V). Thick dilated septa are observed (S). A dilated duct is observed (D). **2B:** showing distorted acini with dark nuclei (arrow) and vacuolated cytoplasm (V). **2C:** showing the islet of Langerhans (I) with a vacuolated cytoplasm (thin arrow) and some cells with no or a karyolytic nucleus (arrow head). Distorted acini are observed (A) with dark nuclei (thick arrow). Dilated duct (D) is observed. (H&E 2A x 100) (H&E 2B,2C x 400).



**Figure3.** A photomicrograph of a section of an adult male albino rat pancreas (withdrawal group). **3A:** showing pancreatic acini with some vacuolations (V), islet of Langerhans (I), and a dilated duct (D). **3B:** showing slightly normal acini (A) with dark nuclei (thick arrow) and other vacuolated acini (V). Islet of Langerhans (I) showing few vacuolations (thin arrow). **3C:** showing islet of Langerhans (I) with few vacuolations (arrow), abnormal dilated duct (D) lined by flat epithelium (curved arrow), and some vacuolated acini (V). (H&E 3A x 100) (H&E 3B,3C x 400).

## DISCUSSION

Energy drink consumption has continued to gain popularity all over the world. These drinks are marketed for the young people as natural alternatives that increase fun and improve physical and cognitive performance such as concentration, attention, and alertness [14].

Red bull has been known as a healthy drink within many populations. Individuals drink red bull to feel energized during the day. The chemical composition of energy drinks can produce multiple adverse effects [15,16].

SOD is important antioxidants which work in association with the nonenzymatic

antioxidant system to protect the cells from the oxidative damage by the free radicals [17]. Definitely, the antioxidant enzymes are the first line of defense which protects the cells from the oxidative stress-induced damage. SOD neutralizes the highly reactive superoxide anion by converting it to the hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) [18].

In the present study, in RB treated group there were a decrease in SOD and GSH levels in the pancreatic tissue. This result was in accordance with that of Mansy *et al.* [19] who gave two oral doses of energy drink (1.1 and 2.2 ml/100g body weight/day) for 12 weeks and reported a significant decrease in SOD. Also,

**Al-Eryani et al. [20]** reported a significant decrease in GSH in homogenized testes after administration of Red Bull and Power Horse for 7 weeks. However, **Ayuob and ElBeshbeishy [21]** revealed a significant decrease in SOD and a significant increase in GSH in the pancreatic homogenate.

A study had shown that exposure of human cells to high levels of caffeine induced a prooxidant environment in the cells, leading to an increased protein oxidation, while low levels of caffeine had no effect on the antioxidant capacity of the cells [22].

In the present study, Oxidative enzymes (SOD and GSH) levels were statistically increased in withdrawal group in comparison to RB treated group. These results were in agreement with **Al-Eryani et al. [20]** who reported a significant increase in GSH level in homogenized testes in withdrawal group in comparison to treated group.

In the present study, examination of the pancreas of the control animals stained with hematoxylin and eosin showed that it's normal architecture. It was consisted of lobules separated by thin connective tissue septa. Each lobule was composed of an exocrine part (acini) and an endocrine part (islets of Langerhans). The acinar cells were pyramidal in shape with rounded basal nuclei with basal basophilic and apical acidophilic cytoplasm. These normal features were similar to those described by **Salam et al. [23]**. Islets of Langerhans appeared as pale oval areas in between the acini. They were composed of groups of cells separated by blood capillaries. Their cells were composed of pale nuclei. These findings are in agreement with that of **Youssef [24]**.

Haematoxylin and eosin-stained Sections of RB treated group revealed massive destructive damage of the pancreas. The pancreatic acini appeared distorted with dark nuclei and intracytoplasmic vacuoles. Pancreatic islets also showed intracytoplasmic vacuoles and Karyolysis, which means disappearance of the nucleus. These findings were in agreement with **Ayuob and ElBeshbeishy [21]** who gave power Horse to

the rats for 4weeks and demonstrated marked histological changes in the pancreatic islets and acini.

The intracytoplasmic vacuoles observed in this study were in agreement with **Mubarak [25]** who observed numerous intracellular vacuolization in the rat submandibular salivary glands after administration of RB for 8weeks. These vacuoles might be due to reduced secretory activity of the acinar cells and decline in the secretory material of the granules.

Haematoxylin and eosin stained-Sections of withdrawal group showed some degrees of improvement compared with RB treated group. The interlobular septa between the pancreatic lobules were dilated and the ducts were slightly dilated. The pancreatic acini appeared slightly normal but some acinar cells still vacuolated with dark nuclei.

We concluded that, in withdrawal group, there was a decrease in the intensity of the histological changes as compared to RB group. These findings may suggest attempts for gland regeneration after withdrawal of the red bull energy drink. These findings were confirmed by **Kassab and Tawfik [26]** who observed an improvement of histological structure of submandibular gland in withdrawal group compared to RB group.

### CONCLUSION

RB produces degenerative changes in the pancreas and causes oxidative stress. Stoppage of RB exposure results in some restoration of the normal histological structure of the pancreas. So, it is recommended to use an antioxidant with RB in further studies.

### Abbreviations

RB= Red Bull

GSH= reduced glutathione

SOD= superoxide dismutase

**Conflict of interest:** No

**Financial Disclosures:** No

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## Cite This Article - VANCOUVER Style

Hulail, M., Qenawy, N., Abdel-Kareem, R., Mohamed, G. The Toxic Effect of Energy Drinks on The Structure of Pancreas of Adult Male Albino Rats. *Zagazig University Medical Journal*, 2020; (1110-1117): -. doi: 10.21608/zumj.2019.11664.1205