Original Article

Structural and Biochemical Changes Induced by Energy Drinks in the Pancreas of Adult Male Albino Rats: Ameliorative Effect of Green Tea

Reham H. Abdel-Kareem, Mohey Elsayed Hulail, Gehad Ahmed Mohamed and Nora Mohamed Qenawy

Department of Human Anatomy & Embryology, Faculty of Medicine, Zagazig University, Egypt

ABSTRACT

Introduction: Energy drinks have been shown to endanger public health and cause a slew of medical issues.

Aim of the Work: Study the histological, and biochemical findings to assess how energy drinks (Red Bull) (RB) affect the rat pancreas and the potential protective role of green tea (GTE).

Material and Methods: Forty adult male albino rats were divided into four groups. 1st group was provided by 7.5 ml of distilled water. 2nd group (GTE) received 200 mg/kg body weight (B.w) of GTE daily for 4 weeks. Rats in the 3th group (RB-administered group) were provided by 7.5 ml of RB divided into two doses daily for 4 weeks. In the 4th group (RB/GTE) rats were provided by 7.5 ml of RB divided into two doses plus 200 mg/kg B.w of GTE daily for 4 weeks. Pancreatic specimens were prepared for histopathological and biochemical examinations.

Results: In RB group, there was significant increase of rats, body weight, serum insulin, glucose and Homeostatic model assessment of insulin resistance (HOMA-IR) levels. Furthermore, there was oxidative damage evidenced by decreased glutathione peroxidase enzyme (GPX) and increased Malondialdehyde (MDA) level in the pancreatic tissue. Histopathologically, RB induced pancreatic distortion with marked increase of collagen fibers around blood vessels and ducts. Immunohistochemically, RB group showed strong positive immune expression of insulin antibody and iNOS. Co-administration of GTE ameliorated these alterations.

Conclusion: RB caused disturbed pancreatic hormonal function, degenerative changes and oxidative stress in the pancreatic tissue. Co-administration of GTE with RB improved these harmful histopathological and biochemical changes.

Received: 14 March 2022, **Accepted:** 06 May 2022

Key Words: Energy-drinks, green-tea, histology, oxidative stress, pancreas.

Corresponding Author: Reham H. Abdel-Kareem, MD, Department of Human Anatomy & Embryology, Faculty of Medicine, Zagazig University, Egypt, **Tel.**: +20 11 4622 2474, **E-mail:** reham.helmy5@gmail.com

ISSN: 1110-0559, Vol. 46, No. 3

INTRODUCTION

Energy drinks (EDs) are non-alcoholic carbonated beverages. These are formulated in small, bullet-shaped cans. They are manufactured to give the consumer an energy jolt via a mixture of vitamin B complex, methylxanthines, and peculiar herbal components[1].

In Egypt, Red Bull is a widely consumed energy drink and its intake said to increase physical and mental performance[2]. Caffeine is the most common ingredient in EDs, although other ingredients include taurine, riboflavin, carbohydrates, vitamin B-complex, nicotinamide, pyridoxine, vitamin B-complex, and natural derivatives like ginseng[3,4]. Overuse of caffeine leads to health problems such as palpitation, hypertension, irritability, insomnia, tremors, and seizures[5]. Synthetic taurine is involved in a number of disorders, including, strokes, seizures, hypertension, and heart diseases. So it was prohibited in some Scandinavian countries, as a result of being related to the deaths of consumers[6]. The high amount of glucose in a can of EDs really leads to overweight and dental caries[7]. Generally, overconsumption of EDs leads to many health problems such as hypertension, cardiac arrhythmia, and toxicity. They also affect the functions of neurons, concentration of catecholamine in the brain, linked to Parkinson's disease and cognitive dysfunction[8]. Hepatotoxicity[9], disturbance of secretory glands[10], nephrotoxicity[11], haematopoietic disorders[12], overweight/obesity hazard and type 2 diabetes mellitus[13], all been linked to EDs. However, a study of the literature revealed that there are little studies on the effects of these beverages on the pancreas.

The most active components of green tea (GTE) are polyphenolic compounds such as catechins which consist of epigallocatechin (EGC), epicatechin (EC), epigallocatechin-3-gallate (EGCG), and epicatechin-3-gallate (ECG)[14]. GTE is a common drink and considered as an antioxidant, anti-inflammatory, anti-cancer, anti-mutation, anti-diabetic, anti-cholesterol, anti-stroke, and antimicrobial substance[15-17]. It has been demonstrated to increase insulin sensitivity and glucose tolerance in healthy humans[18]. It also has therapeutic benefits in neurodegenerative diseases, inflammatory diseases, cardiovascular diseases and several types of cancer[19,20].

Personal non-commercial use only. EJH copyright © 2023. All rights served
DOI: 10.21608/ejh.2022.127251.1657

Based on overconsumption of energy drinks especially in teenagers and young adults, in the hope of gaining a spurt of energy, is emerging questions about safety of this use. So, this present study was conducted for examination of the histopathological and biochemical effects of RB on pancreas of adult male albino rats, as well as for assessing the potential protecting role of GTE against these promising effects.

MATERIAL AND METHODS

Chemicals

Cans of Red Bull were bought from Egyptian markets. The producing company: Fuschl MC Austria. The imported company: Red Bull Egypt for export and import. In each 100 ml of Red Bull there were: taurine (400 mg), caffeine (32mg), glucose and sucrose (11.3 g), gluconolactone (240 mg), niacin (7.2 mg), B12 (0.4mg), B6 (0.8 mg), B2 (0.64 mg), panthenol (2.4 mg), inositol (20 mg), synthetic flavor and sparkly water.

Green tea was provided as tablets of 300 mg/tablet (a packet is 20 tablets), from MEPACO-MEDIFOOD Company for Pharmaceuticals and Medicinal plants, Sharkeya, Egypt. After tablet crushing; the needed dose was weighed on a digital scale and diluted in distilled water.

The study was carried out in accordance with the guidelines of Zagazig University's Institutional Animal Care and Use Committee (ZU-IACUC) and approved in a number of ZU-IACUC/3/F/33/2018. The experiments followed the guidelines and were performed in compliance with the National Institutes of Health's recommendations for the care and use of laboratory animals.

Kits

Assessment of glutathione peroxidase enzyme activity (GPx) and Malondialdehyde (MDA) in the tissue homogenate used Biodiagnostic kits, 29 Tahreer St., Dokki, Giza, Egypt, in accordance with the manufacturer's guildlines (CAT. No. GP 2524) for GPx, and (CAT. No. MD 25 29) for MDA.

Experimental animals

This study was conducted on 40 male adult albino rats (weighing: 210-250 gm). They were attained from Faculty of Medicine's Animal House, Zagazig University. They were kept in sanitary circumstances, fed standard food and drank tap water. They were kept in wide, fan-ventilated polypropylene cages having stainless steel tops and bedding wood shavings. The temperature was preserved at 23±2°C. All rats were kept for 15 days before beginning of experiment, for accommodation with the laboratory conditions. The standard guide for care and use of experimental animals was followed for all rats.

Experimental design

The rats were allocated randomly into 4 groups, 10 rats each: 1st group (control group) was provided by 7.5 ml of distilled water divided into two doses by oral gavage daily for 4 weeks. 2nd group (GTE-administered group) was given 200 mg/kg body weight (B.w) of GTE diluted in distilled water at dose level 50 mg/ 2 ml/rat/day by oral gavage for 4 weeks[21]. 3th group (RB-administered group) was given 7.5 ml of RB divided into two doses by oral gavage daily for 4 weeks[22]. 4th group (RB plus GTE-administered group) was given 7.5 ml of RB divided into two doses plus 200 mg/kg B.w of GTE at dose level 50 mg/2 ml/rat/day by oral gavage for 4 weeks.

The initial body weight of rats in different studied groups was estimated on first day of the experiment before administration of any supplementation using a digital balance. At the end of the experiment, the final body weight was assessed using the same digital balance then all rats were anesthetized by an intra-peritoneal injection of 50 mg/kg B.w of thiopental[23]. Venous blood samples were immediately taken from the retro-orbital plexuses of animals after overnight fasting by means of capillary glass tubes and allowed to clot and centrifuged at 2000 rpm for serum separation and estimation of glucose and insulin levels. Then Laparotomy and careful excision the pancreatic specimens was performed. Samples were divided into 2 parts; one part was processed for histopathological studies and the other was kept at -80 °C for assessment of antioxidant enzyme.

Biochemical studies

Assessment of serum glucose and insulin and estimation of HOMA-IR

Estimation of serum glucose using hexokinase method was according to[24] and estimation of serum insulin using enzyme-linked immuno-sorbent assay (ELISA) was according to[25]. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated, using the formula; HOMA-IR = fasting serum glucose (mg/dL) × fasting serum insulin (μU/mL)/405[26].

Assessment of oxidant/antioxidant activities

Homogenization of pancreatic specimens was carried out using a Teflon–glass homogenizer with 1.15% KCl buffer to get 1:10 (W/V) entire homogenate. Centrifugation of homogenate at 3000 rpm (+4 °C) for 15 min was done for assessment of MDA and GPx according to the method of[27,28] respectively.

Tissue preparation for histological and immunohistochemical analysis

Histological analysis

According to standard procedures[29], pancreatic specimens in the different groups were immediately preserved in 10% neutral buffered formalin and embedded in paraffin. 4-5 μm thickness sections were placed on glass slides, deparaffinized, and then stained with Hematoxylin and Eosin (H&E) and Mallory’s trichrome (MT) stains.

Immuno-histochemical analysis

Immuno-histochemical staining was carried out using Anti-insulin antibody for the appearance of β-cells of Langerhans islets according to the method described by[30] and streptavidin system with antibody against inducible nitric oxide synthase (iNOS) marker for oxidative stress according to[31].

Examination of slides was performed by an electric light microscope (Leica ICC50 W) at the Image Analysis Unit of the Human Anatomy and Embryology Department, Faculty of Medicine, Zagazig University.

Morphometric studies

Assessment of quantitative data was done in 5 different non-overlapped fields (X400) in each slide. From 5 slides, 25 fields for each group were counted. Image analysis software (ImageJ 1.36b; http://rsbweb.nih.gov/ij) was used for estimation of diameter of islets of Langerhans (µm) (the maximum diameter was selected by comparing all possible radii diameters per islet and selecting the greatest), number of β-cells/islet, area% of Mallory trichrome and iNOS and optical density of insulin immuno-staining[32,33].

Statistical analysis

The collected data were carefully coded and analysed with a computer using SPSS (Statistical Package for Social Sciences), version 19 software. Quantitative data were stated as mean ± SD (Standard deviation). In normally distributed data, One-way ANOVA (analysis of variance) test was used for calculation of the difference between quantitative variables in more than two groups. LSD test (Least significant difference) was used for estimating significance between each two studied groups. The *P-value* (significance level) was set at 5%. *P-value* of >0.05 showed non-significant results, *P-value* of <0.05 showed significant results and *P-value* of <0.001 showed highly significant results.

RESULTS

Estimation of initial and final body weights

The initial body weight indicated insignificant difference between different groups. While, the final body indicated high significant increase in RB-administered group and significant increase in RB/GTE group, as compared to the control group. However, RB/GTE group showed high significant decrease in the final body weight, as compared to RB-administered group (Table 1).

Assessment of serum glucose and insulin and estimation HOMA-IR levels in the different groups

Serum glucose, insulin and HOMA-IR levels showed high significant increase in RB-administered and RB/GTE groups, as compared to the control group. However, RB/GTE group revealed high significant decrease, in comparison to RB- administered group (Table 2).

Homogenate pancreatic tissue analysis for oxidant/antioxidant activities in the different groups

Activity of the antioxidant enzyme GPx in the pancreatic tissues showed high significant decrease in RB-administered group and significant decrease in RB/GTE group, in comparison with control group. However, in RB/GTE group, it was high significantly increased, as compared to RB-administered group. MDA content of pancreatic tissues showed high significant increase in RB-administered group and significant increase in RB/GTE group, in comparison with control group. While, in RB/GTE group, there was high significantly decrease in MDA level, as compared to RB-administered group (Table 3).

Histological analysis

Histological examination of pancreas sections using Haematoxylin and Eosin staining

Control group (Figure 1: A,B,C) and GTE-administered group (Figure 1: D,E) showed similar results of normal pancreatic histology; it was divided into well-formed lobules of varying size and shape by thin connective tissue septa. These lobules were closely packed and formed of endocrine part; islets of Langerhans and exocrine part; acini & ducts with predominance of the acinar tissue. Within lobules, Islets of Langerhans were present as a pale stained area in between acini. The interlobular ducts and blood vessels were also seen in the septa between lobules (Figure 1: A,D). The exocrine component of pancreas was made up of closely packed, well-developed serous acini (rounded or oval in shape) with regular distinct acinar boundaries. The pyramidal acinar cells had rounded vesicular basal nuclei, basophilic basal cytoplasm and acidophilic granular apical cytoplasm (Figure 1: B,C,E). The interlobular ducts were lined by cuboidal epithelium (Figure 1: B). Cells of Islets of Langerhans had pale nuclei with blood capillaries in between them (Figure 1: C,E).

RB-administered group showed distortion of the general pancreatic architecture in the form of; separation of pancreatic lobules by dilated interlobular septa (Figure 2: A), pancreatic acini were distorted and showed small darkly stained nuclei and vacuolated cytoplasm and pancreatic ducts were irregular, dilated and lined by flat epithelium with mononuclear cellular infiltration (Figure 2: B). Vacuolations and few cells were present in islets of Langerhans (Figure 2: C). Dilated congested blood vessels with perivascular inflammatory and fat cells were also observed (Figure 2: D).

RB/GTE group exhibited variable degree of improvement in the pancreatic tissue when compared to RB-administered group. It showed apparently normal architecture with thin connective tissue septa (Figure 2: E). Some acinar cells exhibited few vacuolations and darkly stained nuclei. Islets of Langerhans were apparently normal with pale nuclei but few vacuolations were present (Figure 2: F).

Morphometrical evaluation of Langerhans islets, diameter revealed high significant decrease in RB-administered group, as compared to control group. While, RB/GTE group showed significant decrease in comparison with control group and significant increase in comparison with RB/GTE group (Figure 2: G). Number of of β-cells/islet was significantly decreased in RB-administered group, as compared to control group. While, RB/GTE group showed high significant difference, as compared to control and RB-administered groups (Figure 2: H).

Histological analysis of pancreatic sections using Mallory trichorme stain

Mallory's trichrome stained sections of control and GTE-administered groups exhibited scanty blue staining collagen fibers in the connective tissue septa and around ducts and blood vessels (Figure 3: A,B).While, pancreatic sections of RB-administered group showed marked increase of collagen fibers around blood vessels and ducts (Figure 3: C). Pancreatic sections of RB/GTE group showed mild increase in blue staining collagen fibers around blood vessels and ducts (Figure 3: D).

Morphometrical analysis of percentages of Mallory trichrome stain showed high significant increase in RB-administered group, in comparison with control group. While, RB/GTE group showed significant difference, in comparison with control group and RB-administered groups (Figure 3: E).

Immunohistochemical analysis of insulin immuno-staining

Immuno-histochemical stained sections by anti-insulin antibody of control and GTE-administered groups showed positive cytoplasmic reaction (brown color) in the center of the islets (β-cells) (Figure 4: A,B).While, RB-administered and RB/GTE groups showed strongly positive cytoplasmic reaction in the center of the islet (β-cells) (Figure 4: C,D).

Morphometrical analysis of optical density of insulin immuno-staining, showed high significant increase in RB-administered group, as compared to control group. While, RB/GTE group showed significant increase, as compared to control group and significant decrease as compared to RB-administered groups (Figure 4: E).

Immunohistochemical analysis of iNOS immunmostaining

Immuno-histochemical stained sections for iNOS of control and GTE-administered groups showed mild immunoreactivity in the cytoplasm of pancreatic acini and islets (Figure 5: A,B). RB-administered group showed marked immunoreactivity in the cytoplasm of pancreatic acini and islets (Figure 5: C). RB/GTE group showed moderate immunoreactivity in the cytoplasm of pancreatic acini and islets (Figure 5: D). Morphometrical analysis of percentages of iNOS immuno-staining showed significant increase in RB-administered group, in comparison with control group. While, RB/GTE group showed significant increase, as compared to control group and high significant decrease, as compared to RB-administered group (Figure 4: E).

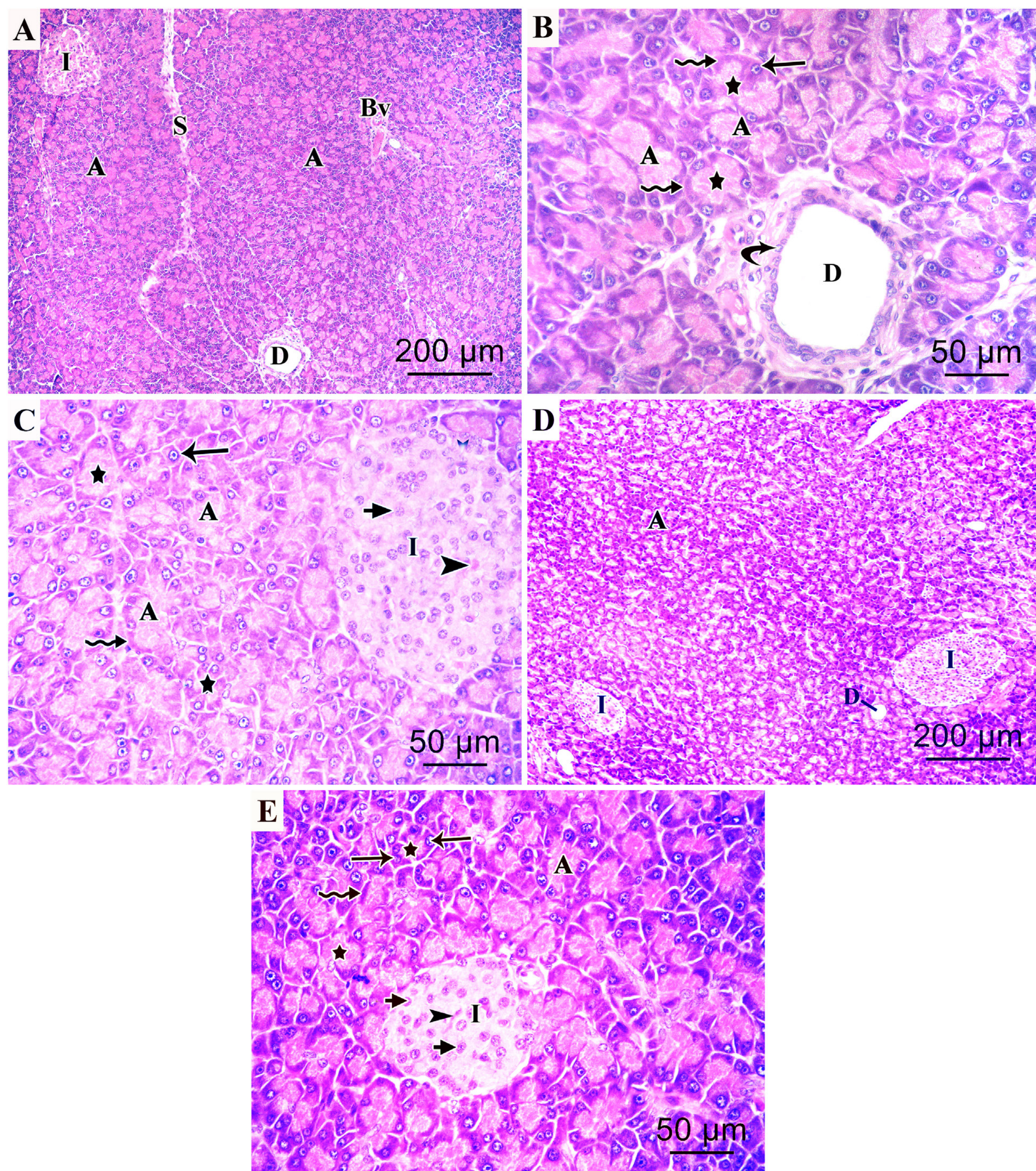


Fig. 1: H&E stained sections of pancreatic tissues of control group (A,B &C) and GTE-adminstered group (D&E) are showing; serous acini (A), thin connective tissue septa (S), pale stained islet of Langerhans (I), interlobular duct (D), and blood vessels (Bv) (A&D). Serous acini (A) are lined pyramidal cells with rounded vesicular basal nuclei (thin arrow), basophilic basal cytoplasm (zigzag arrow) and acidophilic granular apical cytoplasm (star). Ducts (D) are lined by cuboidal epithelium (curved arrow). Cells of Islets (I) are having pale nuclei (thick arrow) and blood capillaries (arrow heads) in between them (B,C&E) (Bar: A&D= 200 µm X 100 & B,C&E=50 µm X 400).

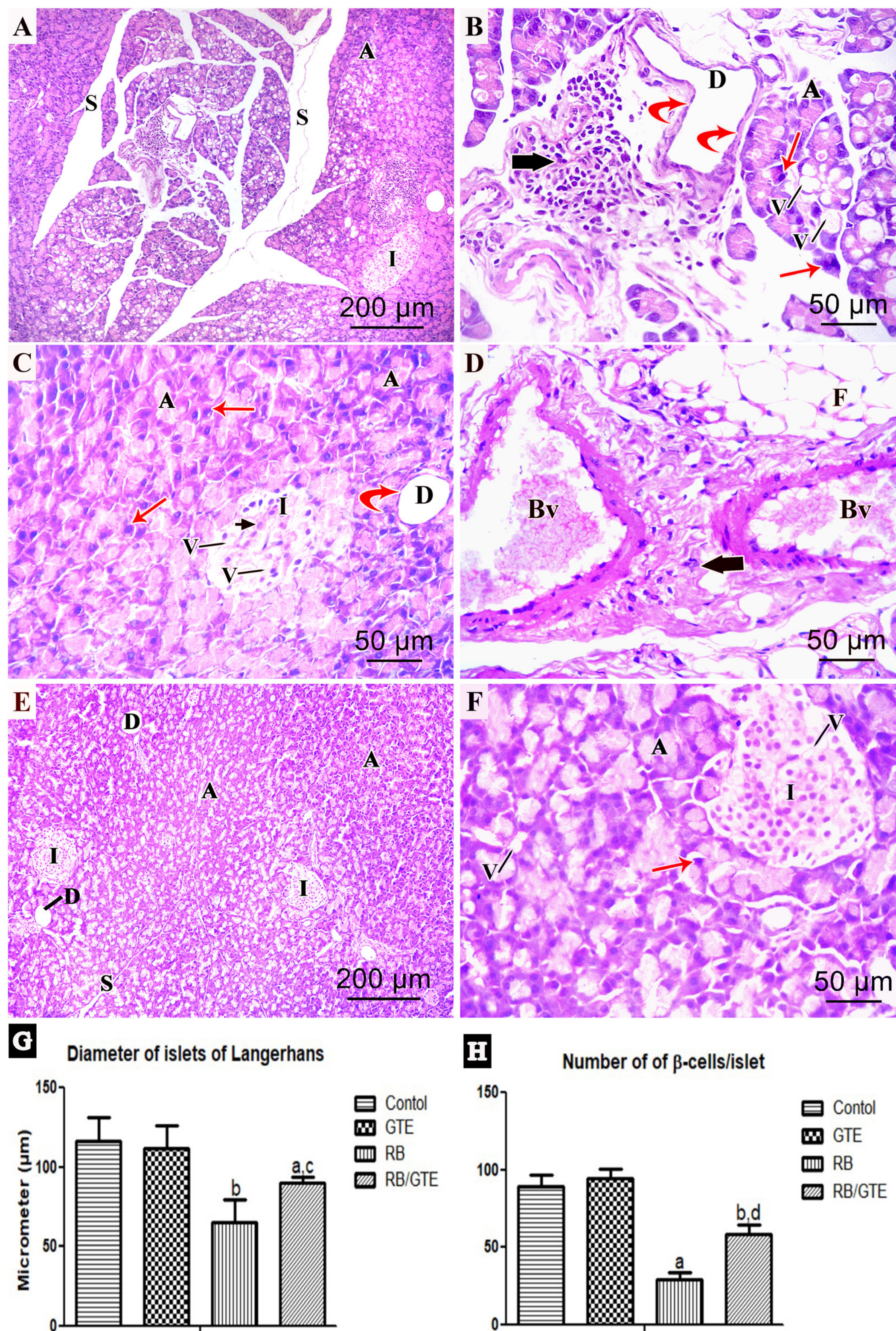
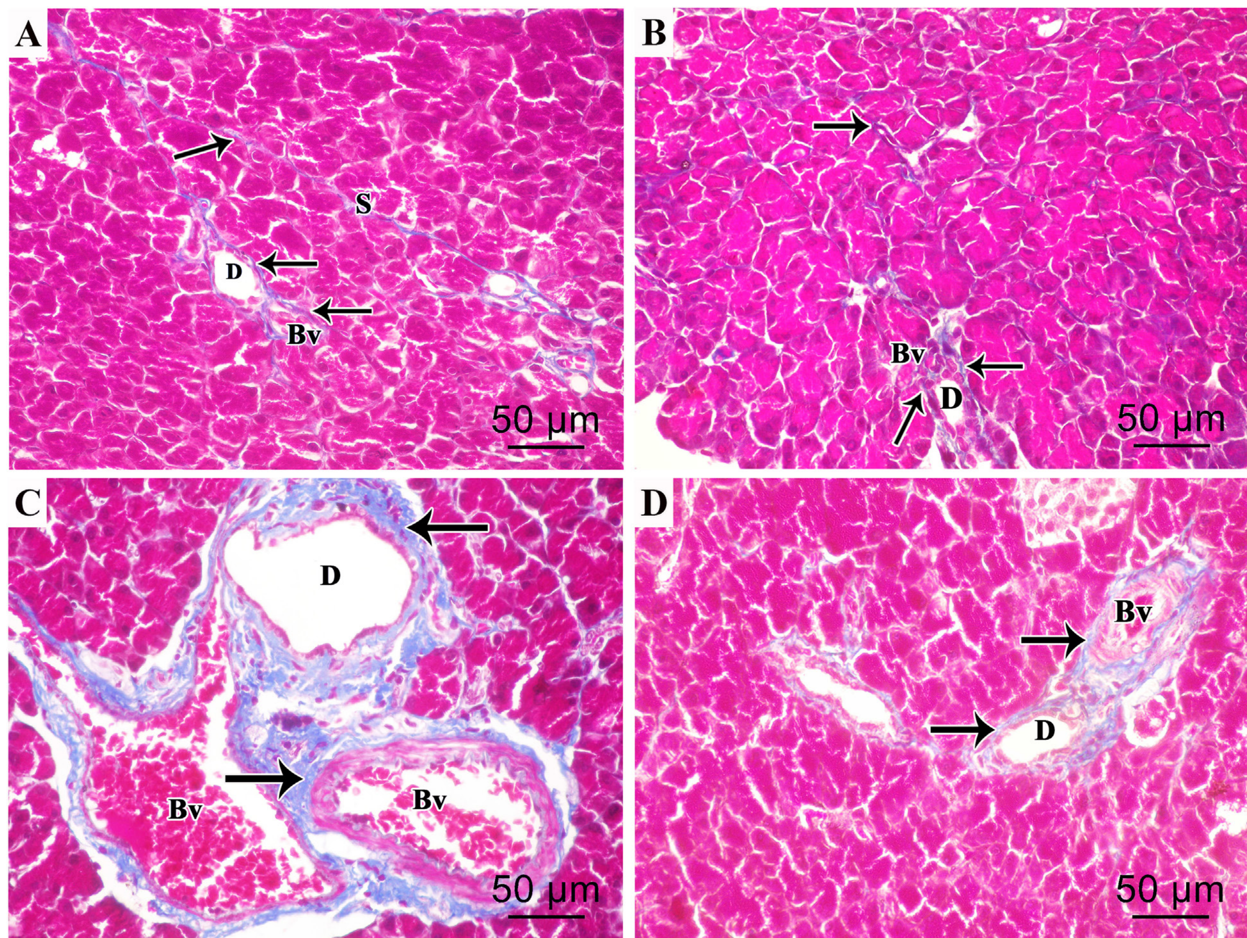


Fig. 2: H&E stained sections of pancreatic tissues of RB-adminstered group (A,B,C&D) are showing; distortion of the general pancreatic architecture in the form of; separation of pancreatic lobules by dilated interlobular septa (S), distortion of pancreatic acini (A) with small darkly stained nuclei (red arrows) and vacuolated cytoplasm (V), irregular dilated pancreatic ducts (D) lined by flat epithelium (red curved arrows) and mononuclear cellular infiltration (thick arrow). The islets of Langerhans (I) are showing vacuolations (V) and few cells (short arrow). Dilated congested blood vessels (Bv) with perivascular inflammatory (thick arrow) and fat cells (F) are also observed. RB plus GTE-administered group (E&F) is showing apparently normal pancreatic architecture, acini (A), ducts (D) and islets of Langerhans (I) with thin connective tissue septa (S) in between. Some acinar cells exhibit few vacuolations (V) and darkly stained nuclei (red arrow). Cells of islets are apparently normal with pale nuclei but few vacuolations (V) are present (Bar: A&E= 200 µm X 100 & B,C,D&F=50 µm X 400). (G&H): Morphometrical comparing of islets, diameter and number of of β-cells/islet between different groups. a: significant vs control group, b: highly significant vs control group, c: significant vs RB group, d: highly significant vs RB group.



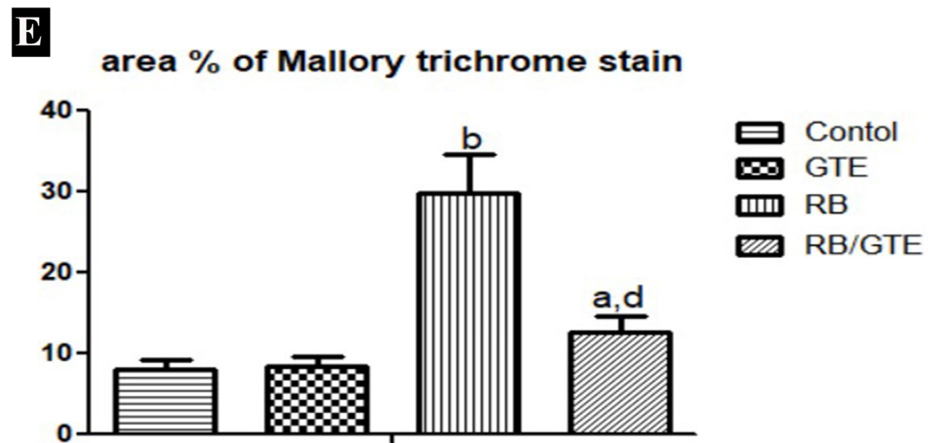
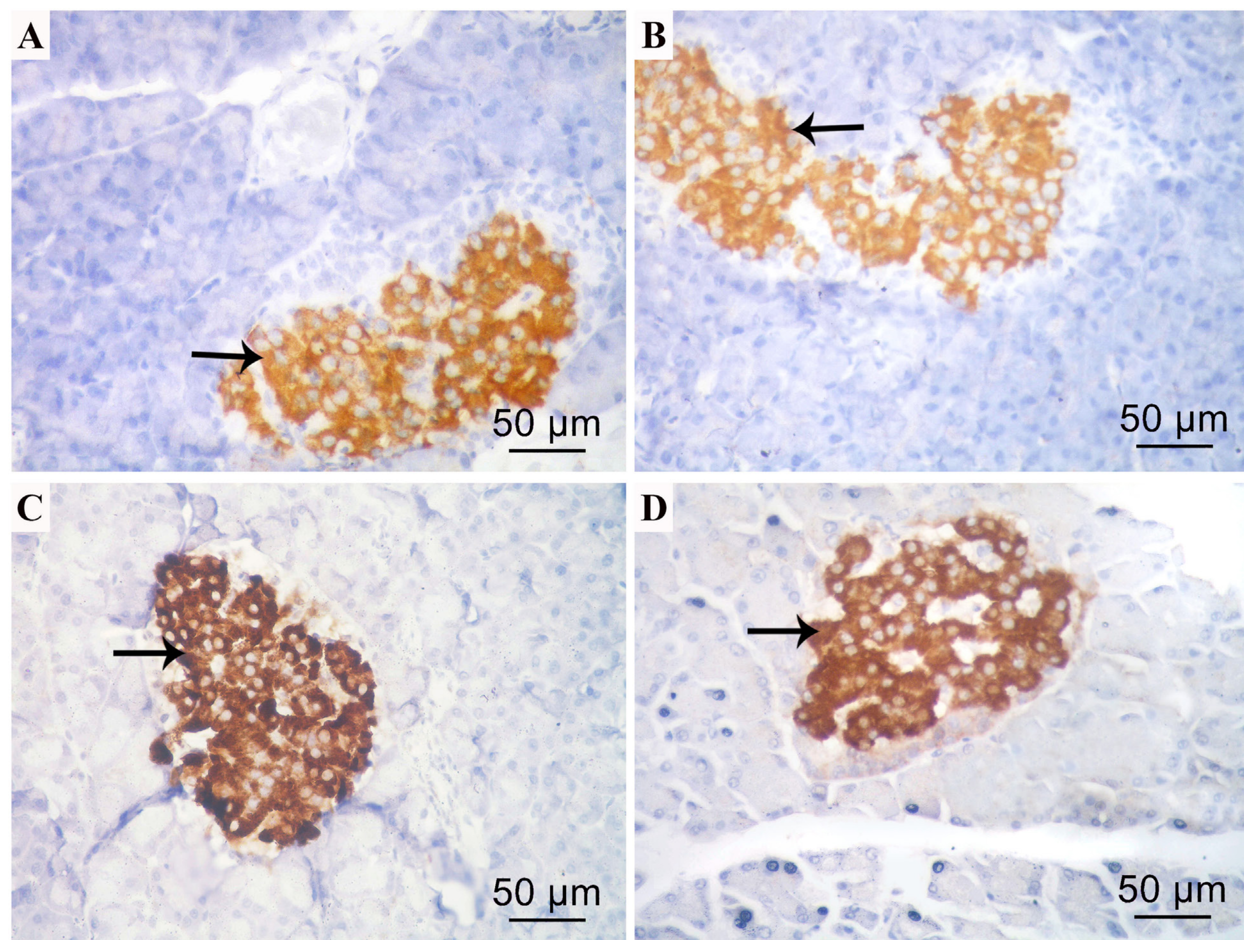


Fig. 3: Mallory's trichrome stained sections of pancreatic tissue are showing blue staining collagen fibers (arrows) in the connective tissue septa (S) and around ducts (D) and blood vessels (Bv). Control group (A), GTE-adminstered group (B), RB-adminstered group (C) and RB/GTE group (D) (Bar= 50 µm X 400). (E): Morphometrical comparing of area% of Mallory trichrome stain in different groups. a: significant vs control group, b: highly significant vs control group, c: significant vs RB group, d: highly significant vs RB group.



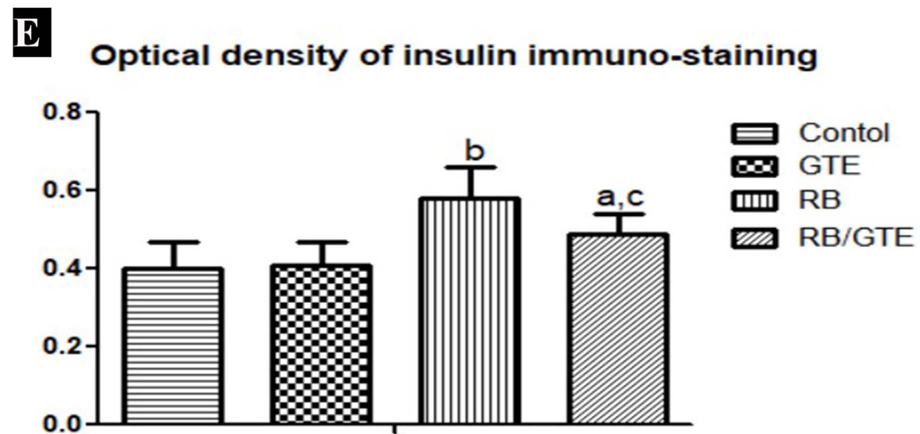
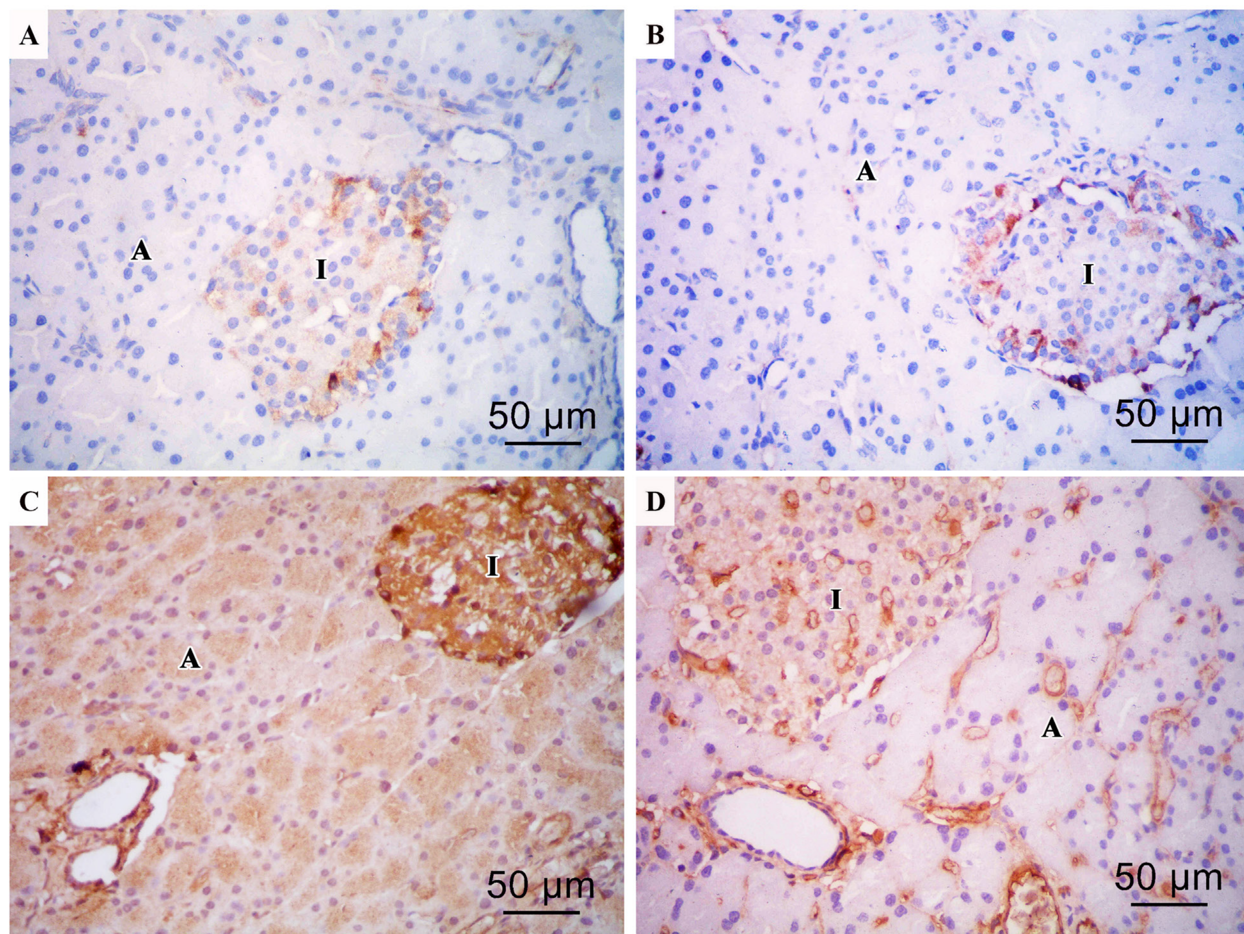


Fig. 4: Immuno-histochemical stained sections of pancreatic tissue by anti-insulin antibody are showing positive cytoplasmic reaction (brown color) in the center of the islets (β-cells) (arrow). Control group (A), GTE-administered group (B), RB-administered group (C) and RB/GTE group (D) (Bar= 50 µm X 400). (E): Morphometrical comparing of optical density of insulin immuno-staining in different groups. a: significant vs control group , b: highly significant vs control group c: significant vs RB group, d: highly significant vs RB group.



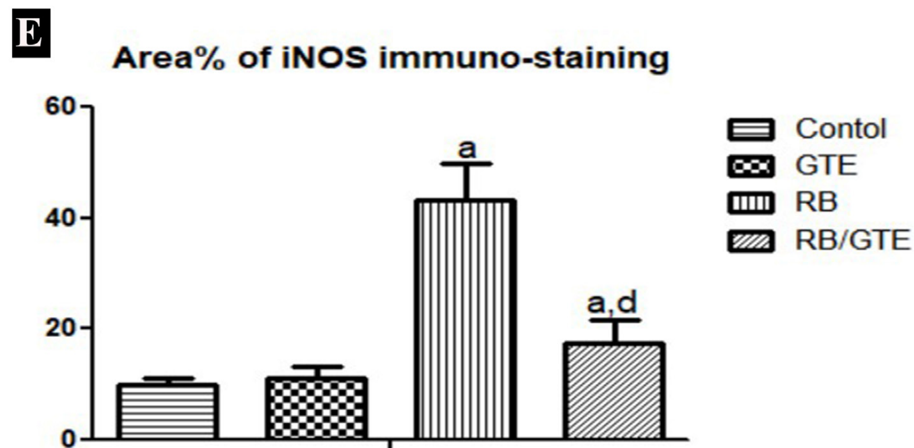


Fig. 5: Immuno-histochemical stained sections of pancreatic tissue by iNOS are showing positive immune reaction (brown color) in the cytoplasm of pancreatic acini (A) and islets (I). Control group (A), GTE-administered group (B), RB-administered group (C) and RB/GTE group (D) (Bar= 50 μm X 400). (E): Morphometrical comparing of area% of iNOS immuno-staining in different groups. a: significant vs control group, b: highly significant vs control group, c: significant vs RB group, d: highly significant vs RB group.

Table 1: Initial and final body weights in the different groups

Group	Control (N=10)	GTE (N=10)	RB (N=10)	RB/GTE (N=10)	*P-value*[1]
Initial BW (gm.)	245± 4.35	246 ± 5.16	243 ± 7.16	247 ± 4.22	0.2939
Final BW (gm.)	362.6 ± 4.62	358.3 ± 3.94	388.4 ± 9.97[b]	271.3± 5.12 [a,d]	0.0000

BW: body weight. [1]One-way ANOVA.

least significant difference (LSD) test were used to show significance between different groups in the same group:
[a] significant vs control group, [b] highly significant vs control group.
[c] significant vs RB group, [d] highly significant vs RB group.
P>0.05: no significant differences, *P*<0.05: significant differences, *P*<0.001: highly significant differences.

Table 2: Serum glucose, insulin and HOMA-IR levels in the different groups

Group	Control (N=10)	GTE (N=10)	RB (N=10)	RB/GTE (N=10)	*P-value*[1]
Glucose:(mg/dl)	107 ± 3.44	102.5 ± 5.99	160.9 ± 10.96[b]	129.3 ± 11.06[b,d]	0.0000
Insulin: (uIU/ml)	4.88 ± 1.2	4.54 ± 0.79	17.21 ± 2.2[b]	12.33 ± 1.5[b,d]	0.0000
HOMA-IR:	1.25 ± 0.21	1.05 ± 0.4	6.7± 1.87[b]	3.8 ± 0.59[b,d]	0.0000

HOMA-IR: Homeostatic model assessment of insulin resistance.
[1]One-way ANOVA.
least significant difference (LSD) test were used to show significance between different groups in the same group:
[a] significant vs control group, [b] highly significant vs control group.
[c] significant vs RB group, [d] highly significant vs RB group.
P>0.05: no significant differences, *P*<0.05: significant differences, *P*< 0.001: highly significant differences.

Table 3: oxidative markers in the different groups

Group	Control (N=10)	GTE (N=10)	RB (N=10)	RB/GTE (N=10)	*P-value*[1]
GPX: (u/g)	270.23±16.2	267.71± 17.47	134.95± 17.96[b]	237.11 ± 16.31[a,d]	0.0000
MDA: (nmol/mg)	20.04±1.84	18.39 ± 2.12	70.64 ± 7.96[b]	45.61 ± 1.38[a,c]	0.0000

GPx: Glutathione peroxidase. MDA: Malondialdehyde. [1]One-way ANOVA.
least significant difference (LSD) test were used to show significance between different groups in the same group:
[a] significant vs control group, [b] highly significant vs control group.
[c] significant vs RB group, [d] highly significant vs RB group.
P>0.05: no significant differences, *P*<0.05: significant differences, *P*< 0.001: highly significant differences.

DISCUSSION

The rising consumption of EDs by teenagers and young adults has gotten attention[34]. They raise blood pressure, heart rate, and glucose levels, interfering with regular sleep patterns[35]. The majority of EDs are deemed dangerous for the human body; consequently, these drinks should be avoided due to their unbalanced components, particularly the added amount of caffeine and sugar[36].

In the present study, the final body weight was significantly increased in RB-administered group. This result was in accordance with Mattioli *et al*.[37] who gave ED orally for 15 days and reported significantly increased body weight in ED-treated animals. They found that body weight gain (BWG) was progressive, overcoming the amount of carbohydrate calories added to the beverages. They explained this BWG by an augmented appetite as shown by the increased consumption of the normal laboratory diet. This anabolic action of EDs can be referred to a digestive effect, direct or mediated, rather than to a simple caloric supplementation. Also, this result was in agreement with human studies, where the intake of sugar-added beverages contributes to weight gain and eventually obesity[38]. However, Bukhar *et al*.[39] and Sadowska[40] reported significantly decreased body weight in ED-group compared with control group. Boyle[41] explained this decrease in the body weight, as EDs are designed to enhance activities and their caffeine content causes anxiety and GIT disturbance. Additinaolly, Ebuehi *et al*.[42], Ayuob and ElBeshbeishy[43], and Schuchowsky *et al*.[44] reported that the final body weight wasn't significantly changed in ED-treated group compared to control group. These results were explained by Nawrot *et al*.[45] who stated that large concentration of caffeine as in EDs has a thermogenic effect responsible for some degree of weight control.

In the present study, the final body weight was high significantly decreased in RB/GTE group in comparison to RB-administered group. These findings confirmed by Pandurangan and Periasamy[46] who stated that, decreased body weight after green tea polyphenol (GTP) administration in acute pancreatitis. Green tea catechins may play a role in prevention of obesity by promoting hepatic lipid metabolism, inhibiting fatty acid synthase, impeding gastric and pancreatic lipases and encouraging thermogenesis[47]. Other studies reported that Epigallocatechin gallate (EGCG) (a component of green tea) impedes lipid absorption through forming complexes with lipolytic enzymes and lipids. Therefore, it interfers

with emulsification, hydrolysis, and subsequent uptake of lipids[48].

In this study, serum glucose, insulin, and HOMA-IR levels were high significantly increased in RB-administered group. These findings matched those of Crisan *et al*.[49] and Sadowska[40] who found hyperglycemia in rats administered EDs for two and six weeks respectively. While, Haroun *et al*.[50] found that serum insulin level was significantly decreased while serum glucose level was significantly increased in RB-administered rats at a dose of 10 mg/kg/day for 4 weeks. Ayuob and ElBeshbeishy[43] also found significantly increased levels of glucose, insulin, and HOMA-IR after rat administration of EDs (power Horse) (PH) for 30 days. Moreover, Combining high sugar or carbohydrate-rich diets with niacin, as in EDs, may affect carbohydrate metabolism and lead to an increase in diabetic cases[51].

Interestingly, in the present study, despite of raised insulin level, the glucose level was also increased. Excessive stimulation of insulin-secreting cells in response to prolonged EDs administration appears to be associated with increasing insulin resistance, as evidenced by a significant rise in HOMA-IR. This hypothesis was explained by Sadowska[40] who indicated that the hyperglycemia and reduced fat content in rat muscles after 6 weeks of EDs consumption was due to metabolic alterations that increased lipolysis and led to the development of insulin resistance. Moreover, caffeine may cause insulin resistance through a variety of mechanisms, including decreased tissue sensitivity to insulin, impaired glucose metabolism, and stimulation of stress hormone secretion, such as adrenaline and cortisol, which increase blood glucose levels, lipolysis, and glucogenesis, as well as lowering peripheral glucose consumption by inactivating key glycolytic enzymes.

In this study, serum glucose, insulin and HOMA-IR levels were high significantly decreased in RB/GTE, as compared to RB- administered group. These findings were supported with Haidari *et al*.[21] who gave the same dose of GTE for the same period and observed significant decrease in the glucose level. Also, Hininger-Favier *et al*.[52] supported significant decrease in the level of serum glucose and insulin when they gave high fructose diet plus green tea to rats for 6 weeks. According to Cao *et al*.[53], EGCG and other catechins aid in the prevention of hyperglycemia by increasing insulin activity. As, GTE controls the expression of genes related in glucose absorption and insulin signaling.

Lipid peroxidation is an autolytic process that results in an inflow of ions and body fluids into the cell, rupturing the cell membrane and causing death. MDA is a highly reactive lipid peroxidation product that functions as a lipid peroxidation marker[54]. MDA levels in tissues are utilized as indicators of oxidative stress and tissue damage[55]. GPx is an essential antioxidant that protects cells from oxidative damage caused by free radicals by working in tandem with the non-enzymatic antioxidant system[56].

In the current study, GPX level in pancreatic tissue there was high significantly decreased in RB-administered group. This finding matched that of Mansy *et al*.[57], who administered two oral dosages of ED (1.1 and 2.2 ml/100g body weight/day) for 12 weeks and found significant decline in GPx levels. As well as, Ayuob and ElBeshbeishy[43] revealed significant decrease in GPx level in pancreatic homogenate.

In this study, in RB-administered group, MDA level was high significantly increased in pancreatic tissue. This was parallel to the results reported by Haroun *et al*.[50] after administration of 10 mg/kg/day of RB for 4 weeks. Moreover, Al-Eryani *et al*.[58] found significant increase in MDA level in homogenized testes after 7 weeks of administration of RB and PH.

In the current study, GPx level was high significantly increased while MDA level was significantly decreased in RB/GTE group, as compared to RB-administered group. These findings were in consistence with Pandurangan and Periasamy[46] who gave a single dose of green tea polyphenol (GTP) for 3 days and stated increase level of GPx and decrease level of MDA in pancreatic tissue. The possible mechanism of these findings was that Green tea catechins (EGCG) can serve as scavengers of reactive oxygen species-induced free radicals, impede lipid peroxidation, and protect cell membranes against oxidative stress[59].

In the present study, H&E stained sections of control group and GTE-administered group showed similar results of normal pancreatic histology. It was divided into well-formed lobules of varying size and shape by thin connective tissue septa. These lobules were formed of endocrine part (islets of Langerhans) and exocrine part (acini & ducts). The pyramidal acinar cells had rounded vesicular basal nuclei, basophilic basal cytoplasm and acidophilic granular apical cytoplasm. These normal structures resembled those indicated by Salam *et al*.[60]. Islets of Langerhans were present as a pale stained area in between acini. Cells of Islets of Langerhans had pale nuclei with blood capillaries in between them. These findings matched those of Youssef[61].

H&E stained sections of RB-administered group showed distortion of general pancreatic architecture. There were separation of pancreatic lobules by dilated interlobular septa, distortion of pancreatic acini and islets, irregular dilated pancreatic ducts, congested dilated blood vessels and mononuclear cellular infiltration. These findings were in agreement with Haroun *et al*.[50] who gave RB for 4 weeks at a dose of 10 mg/kg/day, Ayuob and ElBeshbeishy[42] who gave power Horse for 4 weeks and also, Rehman *et al*.[62] who gave EDs in 2 doses (7.5 ml and 15 ml/day orally, for 4 weeks).

The cellular vacuolations observed in this study were parallel with results of Haroun *et al*.[50]. These vacuoles were also observed in the peripheral blood cells[63] and hepatocytes[22] of adult rats after RB and PH ingestion respectively.

In the current study, H&E stained sections of RB/GTE group revealed that the pancreatic acini and islets of Langerhans were appeared almost normal when compared to RB-administered group, however some acinar and islet cells exhibited few vacuoles. This was parallel to the study of Waer and Helmy[64] who stated that GTE improved the degenerative changes in pancreas induced by oxidative damage of Streptozotocin due to hyperglycemia.

In the present work, Mallory's trichrome stained sections of control group and GTE-administered group displayed few collagen fibers in the connective tissue septa and around ducts and blood vessels. These results were close to those of Taha *et al*.[65]. While, in RB-administered group there was abundant collagen fibers in the connective tissue septa and around ducts and blood vessels. This result was parallel with the study done by Haroun *et al*.[50]. Also, Kassab and Tawfik[66] proved similar observation on submandibular salivary gland after long term consumption of RB. Excessive collagen deposition could be attributed to different active ingredients present in these beverages which cause inhibition of A2A adenosine receptor that protects normal tissues from inflammatory damage. This resulted in development of interstitial inflammation and fibrosis[63,67]. Also, excess fibrosis may be attributable to toxic effect of caffeine that increased fibrin deposition on the connective tissue of rat gingiva[68].

In the present study, Mallory trichrome stained pancreatic sections of RB/GTE group exhibited high significant decrease of collagen fibers in the connective tissue septa and around ducts and blood vessels, in comparison to RB-administered group. Confirmatory to our results, Asaumi *et al*.[69] stated that EGCG prevents production of ethanol-induced type-I procollagen and secretion of collagen so, green tea and polyphenols could stop pancreatic fibrosis by inactivation of pancreatic stellate cell through its antioxidative effect.

In this work, insulin immuno-staining of control group revealed positive immunoreactivity in β-cells cytoplasm which located in the central portion of the islets. This result was in the line with Brom *et al*.[70]. While, RB-administered group showed strong positive immunoreactivity, confirmed morphometrically, as the optical density of insulin immuno-staining was significantly increased, in comparison with control group, and biochemically by elevated insulin level due to excess stimulation of insulin-secreting cells in response to prolonged EDs administration. This result was in agreement with that of Ayuob and ElBeshbeishy[43].

Pro-inflammatory cytokines triggers the production of iNOS, causing overproduction of NO, promoting pancreatic injury[71]. In the present work, iNOS immunohistochemical stained sections of control group revealed mild immunoreactivity in the cytoplasm of pancreatic acini and islets, this is parallel with the study done by Al-Mufti *et al*.[72] and Takahashi *et al*.[73]. While, RB-administered group showed marked immunoreactivity in the cytoplasm of pancreatic acini and islets verified morphometrically and biochemically by increasing oxidative markers as MDA. This result was in the line with Ayuob and ElBeshbeishy[43] who observed increase level of iNOS in pancreatic tissue homogenate. Moreover, RB/GTE group showed moderate immunoreactivity. This result was in agreement with Abd El-Fattah and Ismail[74] who gave 50 mg/Kg GTE orally via gastric tube and observed positive immunoreactivity in the cytoplasm of few hepatocytes in GTE plus ketamine treated group. As, green tea polyphenols decrease (iNOS) gene expression and impede enzyme activity[75].

CONCLUSION

Administration of RB led to pancreatic injury in the form of; disturbed hormonal function and oxidation/antioxidant activities and significant histological and immuno-histochemical alterations with increased of percentages of insulin and iNOS expression. GTE can significantly ameliorate these effects by its anti-inflammatory, antioxidant and anti-diabetic effect.

CONFLICT OF INTERESTS

There are no conflicts of interest.

ACKNOWLEDGEMENTS

Special thanks to Human Anatomy Department, Faculty of Medicine, Zagazig University.

REFERENCES

1. Alford C, Cox H, and Wescott R: The effects of red bull energy drink on human performance and mood. Amino acids. (2001) 21(2): 139-150.
2. Ragsdale FR, Gronli TD, Batool N, Haight N, Mehaffey A, McMahon EC, *et al*.: Effect of Red Bull energy drink on cardiovascular and renal function. Amino acids. (2010) 38(4): 1193-1200.
3. Ferreira SE, Quadros IM, Trindade AA, Takahashi S, Koyama RG, and Souza-Formigoni ML: Can energy drinks reduce the depressor effect of ethanol? An experimental study in mice. Physiology & Behavior. (2004) 82(5): 841-847.
4. Aranda M, and Morlock G: Simultaneous determination of riboflavin, pyridoxine, nicotinamide, caffeine and taurine in energy drinks by planar chromatography-multiple detection with confirmation by electrospray ionization mass spectrometry. Journal of Chromatography A. (2006) 1131(1-2): 253-260.
5. Malinauskas BM, Aeby VG, Overton RF, Carpenter-Aeby T and Barber-Heidal K: A survey of energy drink consumption patterns among college students. Nutrition journal. (2007) 6(1): 1-7.
6. Reissig CJ, Strain EC and Griffiths RR: Caffeinated energy drinks—a growing problem. Drug and alcohol dependence. (2009) 99(1-3): 1-10.
7. Marks R: Energy drinks: A potentially overlooked obesity correlate. Adv. Obes. Weight Manag. Control. (2015) 2: 1-4.

8. Specterman M, Bhuiya A, Kuppuswamy A, Strutton PH, Catley M, and Davey NJ: The effect of an energy drink containing glucose and caffeine on human corticospinal excitability. Physiology & behavior (2005) 83(5): 723-728.
9. Famurewa A, Folawiyo A, Epete M, Onuoha M, Igwe E: Consumption of caffeinated energy drink induces alterations in lipid profile and hepatic aminotransferases in experimental rats. J Chem Pharm Res. (2015) 7: 363-369.
10. Mubarak R: Effect of red bull energy drink on rats submandibular salivary glands (light and electron microscopic study). J Am Sci. (2012) 8(1): 366-372.
11. Khayyat L, Essawy A, Sorour J, and Al Rawi M: Impact of some energy drinks on the structure and function of the kidney in wistar albino rats. Life Sci J. (2014) 11(10): 1131-1138.
12. Olaleru F, and Odeigah P: Effects of energy drink on sperm morphology, haematological parametres and behaviour of adult male mice. Annual Research & Review in Biology. (2015): 288-296.
13. Adjene J, Emojevwe V, Idiapho D: Effects of long-term consumption of energy drinks on the body and brain weights of adult Wistar rats. Journal of Experimental and Clinical Anatomy. (2014) 13(1): 17-20.
14. Tanaka T, Matsuo Y, and Kouno I: Green Tea Polyphenols. Green Tea Polyphenols: Nutraceuticals of Modern Life. (2013): 19.
15. Benelli R, Venè R, Bisacchi D, Garbisa S, and Albini A: Anti-invasive effects of green tea polyphenol epigallocatechin-3-gallate (EGCG), a natural inhibitor of metallo and serine proteases. Biololgical Chemistry. (2002) 383(1): 101-105.
16. Weisburger JH, and Chung FL: Mechanisms of chronic disease causation by nutritional factors and tobacco products and their prevention by tea polyphenols. Food and Chemical Toxicology. (2002) 40(8): 1145-1154.
17. Fujiki H, Suganuma M, Matsuyama S, and Miyazaki K: Cancer prevention with green tea polyphenols for the general population, and for patients following cancer treatment. Current Cancer Therapy Reviews. (2005) 1 (1): 109-114.
18. Venables MC, Hulston CJ, Cox HR, and Jeukendrup AE: Green tea extract ingestion, fat oxidation, and glucose tolerance in healthy humans. The American journal of clinical nutrition. (2008) 87(3): 778-784.
19. Khan N, Afaq F, Saleem M, Ahmad N, and Mukhtar H: Targeting multiple signaling pathways by green tea polyphenol (−)-epigallocatechin-3-gallate. Cancer research. (2006) 66 (5): 2500-2505.
20. Clement Y: Can green tea do that? A literature review of the clinical evidence. Preventive medicine. (2009) 49 (2-3): 83-87.
21. Haidari F, Omidian K, Rafiei H, Zarei M, and Shahi MM: Green tea (Camellia sinensis) supplementation to diabetic rats improves serum and hepatic oxidative stress markers. Iranian journal of pharmaceutical research: IJPR. (2013) 12(1): 109.
22. Akande IS, and Banjoko OA: Assessment of Biochemical Effect of “Power Horse” Energy Drink on Hepatic, Renal and Histological Functions in Sprague Dawley Rats. Annual Research & Review in Biology. (2011) 12: 45-56.
23. Altun MA, Ozaydin A, Arkan H, Demiryas S, Akbas F, Bahtiyar N, and Onaran I: Anesthesia may alter mRNA expression of certain wound healing-associated genes in dermal wound environment of the rats. Molecular biology reports. (2019) 46 (3): 2819-2827.
24. Duxbury M: An enzymatic clinical chemistry laboratory experiment incorporating an introduction to mathematical method comparison techniques. Biochemistry and Molecular Biology Education. (2004) 32(4): 246-249.
25. Webster HV, Bone AJ, Webster KA, and Wilkin TJ: Comparison of an enzyme-linked immunosorbent assay (ELISA) with a radioimmunoassay (RIA) for the measurement of rat insulin. Journal of immunological methods. (1990) 134(1): 95-100.
26. Hamid R, Sayiner ZA, Celekli A, and Araz M: Relationship between Serum Magnesium Level and Insulin Resistance in Obese Non-Diabetic and Diabetic Patients. European Journal of Therapeutics. (2018) 24 (1): 44-48.
27. Ohkawa H, Ohishi N, and Yagi K: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry. (1979) 95(2): 351-358.
28. Paglia DE, and Valentine WN: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of laboratory and clinical medicine. (1967) 70 (1) :158-169.
29. Suvarna KS, Layton C, and Bancroft JD, editors. Bancroft's theory and practice of histological techniques E-Book. Elsevier health sciences. (2018).
30. Sun Q, Nie S, Wang L, Yang F, Meng Z, Xiao H, *et al.*: Factors that affect pancreatic islet cell autophagy in adult rats: evaluation of a calorie-restricted diet and a high-fat diet. PLoS One. 2016 Mar 10;11(3): 1-12.
31. Gealekman O, Abassi Z, Rubinstein I, Winaver J, and Binah O: Role of myocardial inducible nitric oxide synthase in contractile dysfunction and β-adrenergic hyporesponsiveness in rats with experimental volume-overload heart failure. Circulation. (2002) 105(2): 236-243.

32. Shawky LM, Morsi AA, El Bana E, and Hanafy SM: The biological impacts of sitagliptin on the pancreas of a rat model of type 2 diabetes mellitus: drug interactions with metformin. Biology. (2019) 9(1): 6.
33. Pashapoor A, Mashhadyrafie S, and Mortazavi P: Ameliorative effect of Myristica fragrans (Nutmeg) extract on oxidative status and histology of pancreas in Alloxan induced diabetic rats. Folia morphologica. (2020) 79(1): 113-119.
34. Rath M: Energy drinks: what is all the hype? The dangers of energy drink consumption. Journal of the American Academy of Nurse Practitioners. (2012) 24(2): 70-76.
35. Lieberman HR: The effects of ginseng, ephedrine, and caffeine on cognitive performance, mood and energy. Nutrition reviews. (2001) 59(4): 91-102.
36. Gunja N, and Brown JA. Energy drinks: health risks and toxicity. Medical Journal of Australia. (2012) 196 (1): 46-49.
37. Mattioli AV, Pennella S, Manenti A, Puviani MB, and Farinetti A: Influence of energy drinks on obesity: a preliminary experimental study. (2017): 369-372.
38. Berkey CS, Rockett HR, Field AE, Gillman MW, and Colditz GA: Sugar-added beverages and adolescent weight change. Obesity research. Obesity research. (2004) 12(5): 778-788.
39. Bukhar HM, ElSawy NA, and Header EA: Biological effect of high energy drink on normal and hyperglycemic rats. Pakistan Journal of Nutrition. Pakistan Journal of Nutrition. (2012) 11(4): 301-309.
40. Sadowska J: Evaluation of the effect of consuming an energy drink on the concentration of glucose and triacylglycerols and on fatty tissue deposition. A model study. Acta Scientiarum Polonorum Technologia Alimentaria. (2012) 11(3): 311-318.
41. Boyle M: Monster on the Loose-Fueled by its energy drink, Hansen Natural is on a rocket ride. Is the fast-grower due for a crash?. Fortune. (2006) 154(13): 116.
42. Ebuehi OA, Ajayi OE, Onyeulor AL, and Awelimobor D: Effects of oral administration of energy drinks on blood chemistry, tissue histology and brain acetylcholine in rabbits. Nigerian quarterly journal of hospital medicine. (2011) 21(1): 29-34.
43. Ayuob N, and ElBeshbeishy R: Impact of an energy drink on the structure of stomach and pancreas of albino rat: can omega-3 provide a protection?.PloS one. (2016) 11(2): 1-15.
44. Schuchowsky E, Schaefer D, Salvador RA, do Nascimento AE, Til D, Senn AP, and Amaral VL: Effects of energy drinks on biochemical and sperm parameters in Wistar rats. Nutrire. (2017) 42(1): 1-7.
45. Nawrot P, Jordan S, Eastwood J, Rotstein J, Hugenholtz A, and Feeley M: Effects of caffeine on human health. Food Additives & Contaminants. (2003) 20(1): 1-30.
46. Pandurangan AK, and Periasamy S: Modulatory influence of green tea polyphenols on lipid peroxidation during l-arginine induced acute pancreatitis in Wistar rats. IOSR-JESTF. (2013) 3: 77-82.
47. Lin JK, and Lin-Shiau SY: Mechanisms of hypolipidemic and anti-obesity effects of tea and tea polyphenols. Molecular nutrition & food research. (2006) 50 (2): 211-217.
48. Raederstorff DG, Schlachter MF, and Elste V, Weber P: Effect of EGCG on lipid absorption and plasma lipid levels in rats. The Journal of nutritional biochemistry. (2003) 14(6): 326-332.
49. Crisan M, Munteanu C, Rosioru C, and Lang C: Red bull induces biochemical changes in Wistar rat liver. Annals of the Romanian Society for Cell Biology. (2013) 18 (2): 118-122.
50. Haroun H, Mohamed E, El Shahat AE, Labib H, and Atef M: Adverse effects of energy drink on rat pancreas and the therapeutic role of each of bone marrow mesenchymal stem cells and Nigella Sativa oil. Folia morphologica. (2020) 79 (2): 272-279.
51. Zhou SS, Li D, Zhou YM, Sun WP, and Liu QG: B-vitamin consumption and the prevalence of diabetes and obesity among the US adults: population based ecological study. BMC Public Health. (2010). 10 (1): 1-16.
52. Hininger-Favier I, Benaraba R, Coves S, Anderson RA, and Roussel AM: Green tea extract decreases oxidative stress and improves insulin sensitivity in an animal model of insulin resistance, the fructose-fed rat. Journal of the American College of Nutrition. (2009) 28(4): 355-361.
53. Cao H, Hininger-Favier I, Kelly MA, Benaraba R, Dawson HD, Coves S, Roussel AM, and Anderson RA: Green tea polyphenol extract regulates the expression of genes involved in glucose uptake and insulin signaling in rats fed a high fructose diet. Journal of Agricultural and Food Chemistry. (2007) 55 (15): 6372-6378.
54. Ilaiyaraja N, and Khanum F: Amelioration of alcohol-induced hepatotoxicity and oxidative stress in rats by Acorus calamus. Journal of dietary supplements. (2011) 8(4): 331-345.
55. Aitken RJ, Roman SD: Antioxidant systems and oxidative stress in the testes. Molecular mechanisms in spermatogenesis. (2009): 154-171.
56. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, and Rezaie A: Pesticides and oxidative stress: a review. Med Sci Monit. (2004) 10 (6): 141-147.

57. Mansy W, Alogaiel DM, Hanafi M, and Zakaria E: Effects of chronic consumption of energy drinks on liver and kidney of experimental rats. Tropical Journal of Pharmaceutical Research. (2017) 16(12): 2849-2856.

58. Al-Eryani FS, Kelany AM, Amin HA, and Shazly HF: Histological and Physiological Studies on the Effects of Some Energy Drinks on Male Rats. International Journal of Pharmaceutical Research & Allied Sciences. (2018) 7(1): 165-176.

59. Saffari Y, and Sadrzadeh SH: Green tea metabolite EGCG protects membranes against oxidative damage in *vitro*. Life sciences. (2004) 74(12): 1513-1518.

60. Salam GM, Mehlab ME, Shishtawy M, and Zahrani I: Ameliorative effects of selenium and vitamins C and E on chronic fluoride pancreatic toxicity: structural and ultrastructural changes in albino rats. Journal of American Science. (2013) 9 (3): 274-283.

61. Youssef S: Effect of fluoxetine on the pancreas of adult male albino rats and the possible protective role of omega-3: light and electron microscopic study. International Journal of Clinical and Developmental Anatomy. (2017) 3(6): 45-56.

62. Rehman F, Islam ZU, Hameed U, and Rehman S: The effect of energy drink on the pancreas of wistar albino rats–a microscopic study. PAFMJ. (2020) 70(2): 524-528.

63. Khayyat LI, Essawy AE, Al Rawy MM, and Sorour JM: Comparative study on the effect of energy drinks on haematopoietic system in Wistar albino rats. J Environ Biol. (2014) 35(5): 883-891.

64. Waer HF, and Helmy SA: Cytological and histochemical studies on rat liver and pancreas during progression of streptozotocin induced diabetes and possible protection using certain natural antioxidants. The Egyptian Journal of Hospital Medicine. (2012) 48(1): 452-471.

65. Taha NR, Amin HA, and ElBishi ZA: Protective effect of Octreotide on histological and ultrastructural changes in the exocrine pancreas of albino rats induced by L-asparaginase (Elspar). Life Science Journal. (2013) 10(3): 2610-2627.

66. Kassab AA, and Tawfik SM: Effect of a caffeinated energy drink and its withdrawal on the submandibular salivary gland of adult male albino rats: A histological and immunohistochemical study.Egyptian Journal of Histology. (2018) 41(1): 11-26.

67. Tek M, Toptas O, Akkas I, Kazancioglu HO, Firat T, Ezirganli S, and Ozan F: Effects of energy drinks on soft tissue healing. Journal of Craniofacial Surgery. (2014) 25(6): 2084-2088.

68. Takesue M: Effect of caffeine or NaCl on wound healing of the rat gingiva. Light and electron microscopic studies. Aichi Gakuin Daigaku Shigakkai Shi (1989) 27(1): 277-316.

69. Asaumi H, Watanabe S, Taguchi M, Tashiro M, Nagashio Y, Nomiyama Y, Nakamura H, and Otsuki M: Green tea polyphenol (–)-epigallocatechin-3-gallate inhibits ethanol-induced activation of pancreatic stellate cells. European journal of clinical investigation. (2006) 36(2): 113-122.

70. Brom M, Joosten L, Frielink C, Boerman O, and Gotthardt M: 111In-exendin uptake in the pancreas correlates with the β-cell mass and not with the α-cell mass. Diabetes. (2015) 64(4): 1324-1328.

71. Rahman SH, Ammori BJ, Larvin M, and McMahon MJ: Increased nitric oxide excretion in patients with severe acute pancreatitis: evidence of an endotoxin mediated inflammatory response?. Gut. (2003) 52(2): 270-274.

72. Al-Mufti RA, Williamson RC, and Mathie RT: Increased nitric oxide activity in a rat model of acute pancreatitis. Gut. (1998) 43(4): 564-570.

73. Takahashi M, Kitahashi T, Ishigamori R, Mutoh M, Komiya M, Sato H, *et al*.: Increased expression of inducible nitric oxide synthase (iNOS) in N-nitrosobis (2-oxopropyl) amine-induced hamster pancreatic carcinogenesis and prevention of cancer development by ONO-1714, an iNOS inhibitor. Carcinogenesis. (2008) 29(8): 1608-1613.

74. Abd El-Fattah LI, and Ismail DI: Histological study on the protective effect of green tea extract on the liver of rats exposed to ketamine. Journal of Cytology & Histology. (2015) 6 (5): 349.

75. Ibrahim AA, Saleh HE, and El-Shinnawy NA: The Role of Ginger or Green Tea in Counteracting the Deleterious Effects of Benzene Sulfonic Acid in Weanling Male Rats. Egyptian Journal of Natural Toxins. (2008) 5: 56-99.

الملخص العربى

التغيرات التركيبية والبيو كيميائية التي تحدثها مشروبات الطاقة في بنكرياس ذكور الجرذان البيضاء البالغة: التأثير التحسيني للشاي الأخضر

ريهام حلمى عبدالكريم، محيي السيد هليل ، جهاد احمد محمد ، نورا محمد قناوي

قسم التشريح الآدمي والأجنة، كلية الطب البشرى، جامعة الزقازيق، مصر

المقدمة: ثبت أن مشروبات الطاقة تعرض الصحة العامة للخطر وتتسبب في عدد كبير من المشكلات الطبية.
الهدف من الدراسة: دراسة النتائج النسيجية والكيميائية الحيوية لتقييم كيفية تأثير مشروبات الطاقة (ريد بول) على بنكرياس الجرذان والدور الوقائي المحتمل للشاي الأخضر.
المواد والطرق المستخدمة: تم تقسيم أربعين من ذكور الجرذان البيضاء إلى أربع مجموعات. أعطيت المجموعة الأولى ٧,٥ مل من الماء المقطر. المجموعة الثانية تلقت الشاي الأخضر ٢٠٠ مجم / كجم من وزن الجسم يوميا لمدة ٤ أسابيع. وأعطيت الفئران في المجموعة الثالثة (المجموعة التي تتناول ريد بول) بمقدار ٧,٥ مل من ريد بول مقسمة إلى جرعتين يومياً لمدة ٤ أسابيع. أما المجموعة الرابعة تم إعطاءها ٧,٥ مل من ريد بول مقسمة إلى جرعتين بالإضافة إلى الشاي الأخضر ٢٠٠ مجم / كجم من وزن الجسم يومياً لمدة ٤ أسابيع. وتم تحضير عينات البنكرياس للفحوصات النسيجية والبيو كيميائية.
النتائج: في مجموعة الريد بول ، كانت هناك زيادة في وزن جسم الفئران والأنسولين في الدم والجلوكوز ومستويات HOMA-IR. علاوة على ذلك، كان هناك ضرر مؤكسد يتضح من انخفاض إنزيم الجلوتاثيون بيروكسيديز وزيادة مستوى Malondialdehyde في أنسجة البنكرياس. من الناحية النسيجية ، فقد تسبب الريد بول في تشوه البنكرياس مع زيادة ملحوظة في ألياف الكولاجين حول الأوعية الدموية والقنوات. من الناحية المناعية ، أظهرت مجموعة الريد بول تعبيرًا مناعيًا إيجابيًا قويًا للأجسام المضادة للأنسولين و iNOS. بينما تناول الشاي الأخضر خفف من هذه التغيرات
الخلاصة: تسبب الريد بول في تغيرات تنكسيه وإجهاد مؤكسد في أنسجة البنكرياس. كما أدي الإدارة تناول الشاي الأخضر مع الريد بول إلى تحسين هذه التغيرات النسيجية والبيو كيميائية الضارة.