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# Histological and Immunohistochemical Investigation Concerning the Potential Protective Function of Quercetin and Omega-3 against the Detrimental Effects of Energy Drinks on The Pancreas of Adult Male Albino Rats

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## ABSTRACT

**Background:** Controversy and investigation surround the global popularity of energy drinks concerning their possibly damaging health impacts. **Objective:** To assess if quercetin and omega-3 have any protective actions on the hazards of energy drink on pancreatic tissue.

**Materials and methods:** Five groups, 10 adult male albino rats each, were created; the group I (control), group II received 7.5 ml of Red Bull daily orally, group III was given Red Bull alongside quercetin daily, group IV was given daily Red Bull combined with omega-3 a and group V received Red Bull combined with both quercetin and omega-3. The experiment lasted 4 weeks. The pancreatic samples were examined immunohistochemically, biochemically, and histologically. The results were subjected to morphometric and statistically analysis.

**Results*:*** Red Bull administration elevates Malondialdehyde (MDA) and lower superoxide dismutase (SOD) and Glutathione (GSH) significantly in comparable to control group. In H&E-stained sections, Red Bull induced impairment in pancreatic tissue; revealed several deteriorations of acini, a marked infiltration of inflammatory cells and vacuolations between acini. In Masson-stained sections, Red Bull exhibited extensive collagen fibers infiltration around the islets of Langerhans and between acini and also demonstrated significant positive immunoreactions. Administration of either quercetin and omega-3 in group III and IV showed a moderate improvement histologically and chemically while administration of both materials together in group V significantly ameliorated the pancreatic alterations induced by Red Bull. **Conclusion:** Quercetin and omega-3 fatty acids, when administered together, protect the pancreatic tissue from the negative effects of energy drinks.

**Keywords:** Energy Drinks, Quercetin, Omega-3, Pancreas.

## INTRODUCTION

Energy drinks (Eds), which are promoted as activators having memorable titles that convey speed, strength, and power, are produced by a variety of competing companies. The active ingredients in the majority of Eds have a major impact on the metabolic and mental energy production of the human body, which enhances activity and focus when driving and studying, combats drowsiness, and lessens headache symptoms [1]. Besides their detrimental effects on the hematopoietic, cardiovascular, and neurological systems, numerous investigations have demonstrated that they also have adverse impacts on the liver and kidney [2]. A flavonoid that occurs naturally, quercetin (QR) is found in many fruits and vegetables, with onions containing the highest concentration [3]. The favorable effects of quercetin are attributed to its direct ability to scavenge free radicals and its indirect enhancement of endogenous antioxidant synthesis [4].

With omega-3 fatty acid, which includes long-chain omega-3 fatty acids with appealing names that convey strength, power, and speed, is one type of polyunsaturated fatty acid, as the two acids are eicosapentaenoic and docosahexaenoic. These are usually found in fish, while alpha-linolenic acid is located in plant oils like flaxseed, rapeseed, and canola oil [5]. It has been demonstrated that omega-3 polyunsaturated fatty acids (PUFA) exhibit bioactive properties linked to their well-known anti-inflammatory advantages [6]. The study's objective was to determine if energy drinks could injure adult male albino rats' pancreas and if quercetin and omega-3 fatty acids could have a protective value.

## MATERIALS AND METHODS

### Animals

We purchased fifty adult Wistar albino male rats, 200–250 grams in weight, from the Breeding Animal House at the Faculty of Medicine, Zagazig University, Egypt. Under typical laboratory conditions, they were housed at room temperature in stainless-steel cages for acclimation. Throughout the experiment, they had unrestricted access to water and standard laboratory meals. This experiment was performed in Anatomy Department, Benha Faculty of Medicine in the period from september 2024 to october 2024.

### Chemicals:

**The energy drink:** Red Bull (RB) GmbH (5330 Fuschl am See, Austria), is sold in 250 ml cans in Egypt. According to the product company's label, each 100 ml container contains the following ingredients: water, sugar, glucose, sodium citrate, niacin (8 mg), pantothenic acid (2 mg), carbon dioxide, taurine (0.4%), caffeine (0.03%), gluconolactone (0.24%), inositol, vitamin B6 (2 mg), vitamin B12 (0.002 mg), caramel,



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riboflavin, and both fake and natural flavorings and colorings. These are the product company's listed ingredients on the cans.

**Quercetin (QR)**: The source of quercetin powder was Sigma-Aldrich Chemical Company (St. Louis, Missouri, USA), in the form of a yellow powder and was dissolved in distilled water

**Omega-3:** Was offered in a 120 ml liquid syrup form. Its ingredients per 5 milliliters: Dl-alpha tocopherol acetate, thyme oil 0.40 mg, Rigel evening primrose oil 213 mg, and high DHA fish oil 640 mg; are all equal to 7.82 mg of vitamin E, produced by Sigma Pharmaceutical Industries.

**Experimental design:** The rates were split up into 5 groups, each with ten rats, and they were given the medication once a day for four weeks.

- **Group I (control group)**: For four weeks, ten rats were given 7.5 milliliters of regular saline orally every day.
- **Group II (RB treated group):** Ten rats each received a dose of 7.5 ml of Red Bull orally for four weeks, via a stomach tube once every day [7].
- **Group III (Quercetin and RB):** For four weeks, ten rats were administered 50 mg/kg/day of quercetin via a gastric tube once daily in addition to Red Bull at the same dosage as in group II [8].
- **Group IV (Omega-3 and RB group):** Comprised 10 rats that were administered Red Bull at the same dosage as group II and 300 mg/kg of omega-3 daily via a gastric tube once a day for four weeks [9].
- **Group V** (**Quercetin+ omega-3 plus RB group**): Comprised 10 rats that were given Red Bull at the same dosage as group II, along with 50 mg/kg of quercetin and 300 mg/kg of omega-3 per day via a stomach tube once a day for four weeks.

***Sample Collection:***

Sodium thiopental (40 mg/kg) was injected intraperitoneally to euthanized the animals 24 hours after the last dose[10]. A mid-ventral incision was made along the full length of the abdominal cavity in each rat to expose the abdominal wall, and the pancreas was removed. The duodenum, gastric, and splenic segments make up the rat pancreas. There are more islets in the splenic segment than in other areas [11]. For light microscopy, specimens primarily from the pancreatic splenic section were acquired and prepared.

**Biochemical analysis:**

Tissue level of Glutathione (GSH ) in pancreas tissue was measured according to the method reported by **Weydert and Cullen [12]**. Additionally, superoxide dismutase (SOD) activity was assessed as indicated. by **Nishikimi technique [13]**. Malondialdehyde (MDA) which is a lipid peroxidation indicator was assessed in pancreatic homogenate [14].

All the above used kits were provided by Bio diagnostic, Cairo, Egypt (Catalog Number: MD 25 29, Catalog Number: GR 25 11, Catalog Number: SD 25 21, correspondingly).

**Light microscopic examination**

The pancreatic specimens were cleaned, dehydrated, clarified, and embedded in paraffin after being submerged in 10% neutral-buffered formalin. Masson Trichrome stain and Haematoxylin and Eosin (H&E) were used to stain 5 μm-thick pancreatic slices [15].

**Immunohistochemical staining**

Utilizing caspase-3 for revealing of apoptosis:

After being deparaffinized, rehydrated, and rinsed with phosphate buffered saline (PBS), pancreatic slices measuring 5 μm in thickness were incubated with 10% normal goat serum in PBS. Following an overnight incubation period at 4°C in a humid chamber with the primary antibodies, the sections were incubated with biotinylated goat anti-rabbit IgG for 60 minutes at room temperature and then with a streptavidin–biotin–horseradish peroxidase complex for an additional 60 minutes. The primary antibodies were rabbit polyclonal antibody anti-active caspase-3 (ab2302; Abcam, Cambridge, Massachusetts, USA). Sections were counterstained with Mayer's haematoxylin after the immunoreaction was visualized using 3,3′-diaminobenzidine (DAB) hydrogen peroxide chromogen. The primary antibodies were left out of the negative control sections [16]. Jurkat cells treated with camptothecin served as positive controls for activated caspase-3. The active caspase-3- immune-stained pancreatic sections were considered positive when expressing clear evident brown nuclear and/or cytoplasmic coloration.

***Morphometric analysis***

A Leica light microscope (DM500, Switzerland) coupled to a Leica digital camera (ICC50, Switzerland) was used for image acquisition and the software "ImageJ" (version 1.48v National Institute of Health, Bethesda, Maryland, USA) was used for the analysis of images. From each segment, ten distinct non-overlapping fields were chosen at random and analyzed to quantitatively assess:

1. At a 200x magnification, the average area % of collagen fibers.
2. At a 400x magnification power, the typical area proportion of immunohistochemically detected positive active caspase-3.

**Ethical consideration: Every experimental procedure followed Official Animal Care and Practice Committee's recommendations and received approval from Benha Faculty of Medicine, Benha University, Egypt, Code number RC 2-8-2024.**

***Statistical analysis***

Version 20 of the Statistical Package for the Social Sciences (SPSS) software (SPSS Inc., Chicago, Illinois, USA) was used to analyze the data that had been gathered. If the P value was ≤ 0.05, it was deemed significant and if it was ≤ 0.001 it was considered

extremely significant. The mean ± SD was used to express all data.

**RESULTS**

**Oxidative stress indicators:**

Compared to group I, group II presented a considerable rise in tissue MDA levels and a concurrent decline in tissue GSH and SOD. according to the findings in Table 1. groups III, IV, and V demonstrated significantly greater levels of SOD and GSH and notably lower levels of MDA compared to group II, but non-significant versus control group (Table 1).

**Table 1.** Comparisons of the levels of oxidative markers in the several groups under investigation using ANOVA (analysis of variance) test (Mean ±SD)

| **Variable** | Group I Control (n=10) | Group II RB (n=10) | Group III Quercetin + RB (n=10) | Group IV Omega-3 + RB(n=10) | Group V Quercetin+ omega-3 + RB(n=10) |
|---|---|---|---|---|---|
| SOD: (u/g) *Mean ± SD* | 4.84 ± 0.02 | 3.22 ± 0.05 [a, c, d& e**] | 3.78 ± 0.04 [b*] | 3.89 ± 0.04 [b*] | 4.05 ± 0.03 [b*] |
| GSH: (nmol/g) *Mean ± SD* | 6.42 ± 0.16 | 3.88 ± 0.05 [a, c, d& e**] | 4.56 ± 0.15 [b*] | 4.68 ± 0.18 [b*] | 4.88 ± 0.18 [b*] |
| MDA of Pancreas (nmol/mg *Mean ± SD* | 15.02±0.49 | 26.9±0.4 [a, c, d& e**] | 20.92±1.1 [b*] | 18.51±0.49 [b*] | 16.56±0.49 [b*] |

SOD: Superoxide dismutase, GSH: Glutathione, MDA: Malondialdehyde. U/g: microgram ,nmol: number of moles. n: number, SD: standard deviation

**P**: p-value**. *:** significant ($p \leq 0.05$). ****:** highly significant ($p \leq 0.001$).

Values are presented as mean ± SD

a: significantly highly different with the control group at ($p<0.001$).

b: significantly different with the RB group at ($p < 0.05$).

c: significantly different with the group III ($p < 0.05$).

d.: significantly different with group IV. ($p < 0.05$).

e: significantly different with V group ($p < 0.05$).

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**Light Microscopic Examination:**

**Histopathological examination:**

The H&E-stained pancreatic slices of a male adult rat of the control group demonstrated that the gland was separated into well-developed pancreatic lobules of varying size and shape by thin connective tissue septa. These lobules, which were composed of an endocrine component (islets of Langerhans) and an exocrine component (acini and ducts), were closely packed together and divided by thin connective tissue septa. The majority of the tissue was acinar. The islets of Langerhans showed up as a pale, stained patch between the acini and were embedded within the lobules. Serous acini of normal shape and appearance made up the exocrine part of the pancreas and Langerhans, which were rich in capillaries. They looked round or oval in shape, were densely packed, and were well developed. Basal pale elliptical nuclei, secretory granule-filled apical acidophilic cytoplasm, and basal basophilic cytoplasm were the characteristics of pyramidal acinar cells. The cuboidal epithelium was observed lining the ducts **(Fig. 1a)**. Group II revealed several deteriorated acini, a large infiltration of inflammatory cells, many vacuolations appeared between acini, and collagen fibers surrounding arteries and the islets of Langerhans. Additionally, endocrine cell death was seen in numerous vacuolations, additionally there were clogged blood vessels between acini and in the islets of Langerhans. **(Fig. 1b).**

The exocrine acinar cells appeared normal with slight inflammatory cellular infiltration were observed in the pancreatic sections of group III, which also demonstrated an improvement in the islets of Langerhans, with the exception of a few vacuoles that were visible. The blood arteries remained clogged. **(Fig 1c)** In group IV, the islets of Langerhans improved, displaying a large number of normal exocrine acinar cells with modest inflammatory cellular infiltration between acini. Congested additionally, blood vessels were seen in the islets of Langerhans and between acini **(Fig. 1d).** However, group V's histology pancreatic tissue revealed a noticeable recovery, with almost normal islets of Langerhans and mildly clogged blood arteries encircled by what is likely typical exocrine architecture of the pancreatic tissues' acinar cells **(Fig. 1e)**.

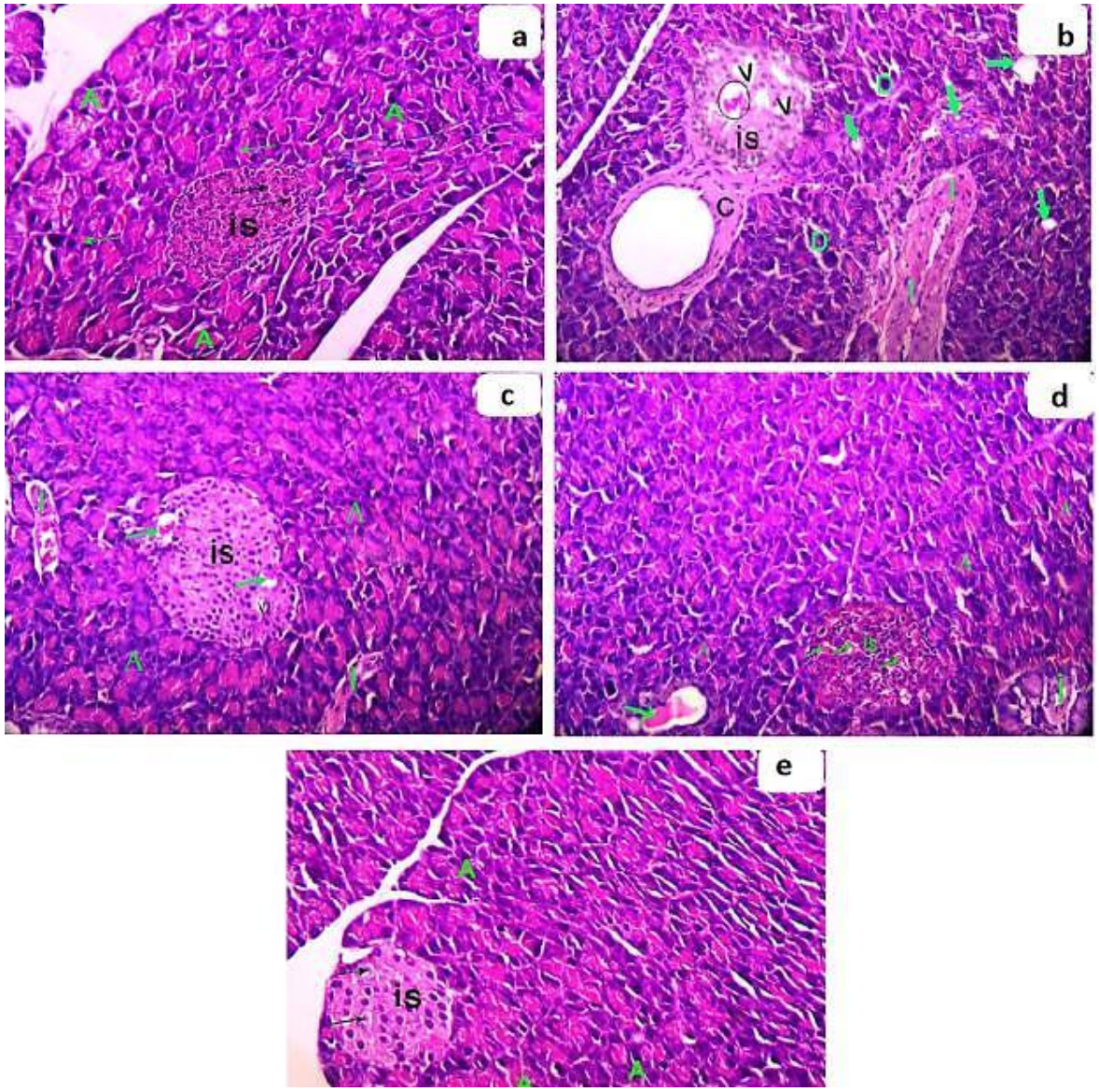


**Figure 1.** A photomicrograph of a slice of pancreatic tissue of albino rats**: [a]** Control group (group I) demonstrating: Normal exocrine acinar constructing of closely packed acini (A) having granules of zymogen in their cytoplasm (green arrows) with normal islets of Langerhans (is) rich in capillaries (black arrows). **[b]** Group II demonstrating: Collagen fibers (C) next to the islets of Langerhans (is), numerous degraded acini (D), a large infiltration of inflammatory cells (i), and vacuolations (arrows) between acini. Additionally, the Langerhans islets' vacuolations (V) and clogged blood vessels (circle) indicate the death of endocrine cells. **[c]:** Group III demonstrating: Congested blood arteries (arrows) and modest vacuolation (V) in the islets of Langerhans (is) indicate an improvement in the organs. They had minor inflammatory cellular infiltration (i) and were encircled by a large number of pancreatic tissues' typical exocrine acinar cells (A). **[d]:** Group IV demonstrating: Enhancement of the Langerhans Islets (is) with many normal exocrine acinar cells (A) with mild inflammatory cellular infiltration in between acini (i). Also, there are engorged blood vessels (green arrows) between acini and in islets of Langerhans**. [e]:** Group V demonstrating: Significant amelioration of the islets of Langerhans (is), with mildly clogged blood arteries (arrows) encircled with what is most likely the typical exocrine architecture of the pancreatic tissues' acinar cells (A). (H&E x 200).

**Masson's trichrome stain results**

There were fine collagen fibers visible between the pancreatic acini and around the islets of Langerhans in control group pancreatic slices stained with Masson's dye **(Fig. 2a)**. Group II, nonetheless, revealed a notable quantity of collagen fiber deposition between the pancreatic acini and blood vessels as well as around the islets of Langerhans **(Fig. 2b).** Collagen fibers were seen in mild to moderate levels in group III, around the islets of Langerhans and in between the blood arteries and pancreatic acini **(Fig. 2c).** Group IV showed moderate quantities of collagen fibers between the pancreatic acini and blood arteries, as well as around the islets of Langerhans **(Fig. 2d).** Ultimately, Collagen fibers were found in very little levels between the pancreatic acini in group V. and blood vessels, as well as surrounding the islets of Langerhans **(Fig. 2e).**

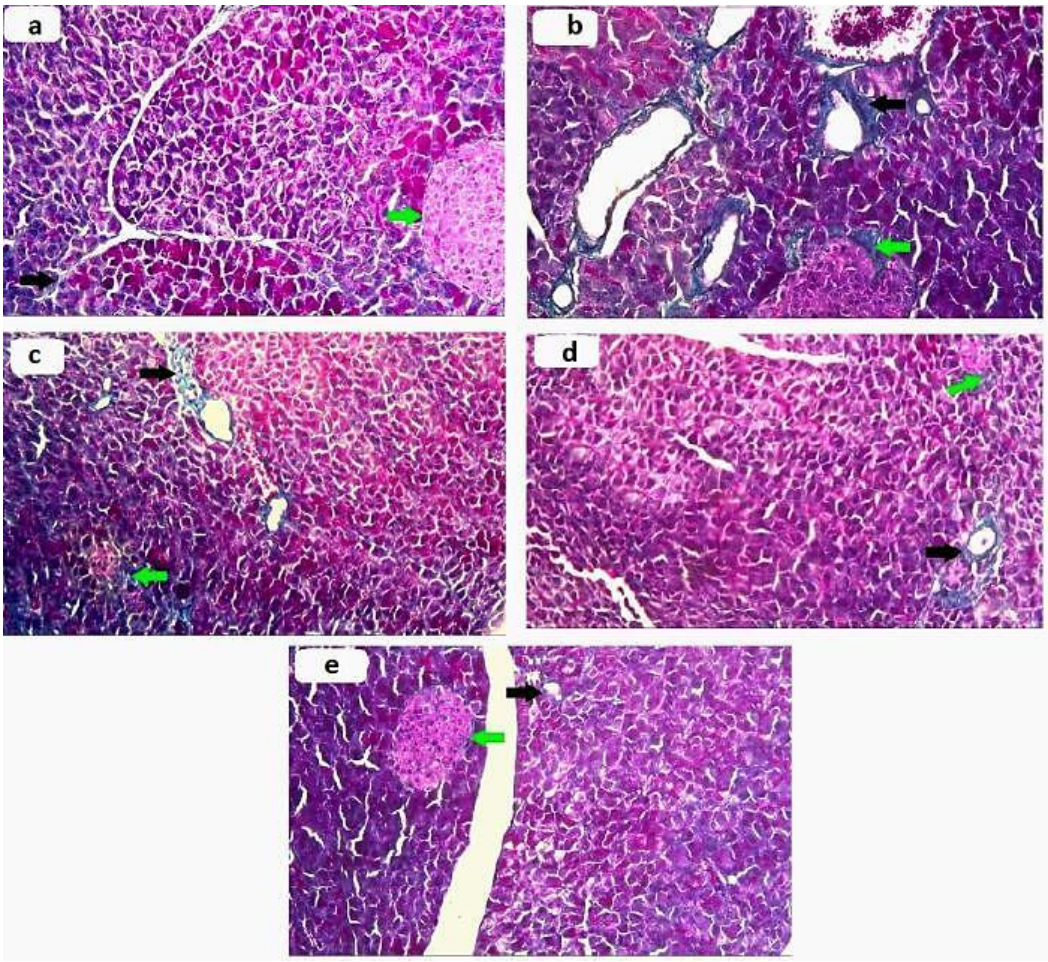


**Figure 2.** A photomicrograph of a slice of pancreatic tissue taken from albino rats showing: **[a]** Group I (control group) demonstrating very little collagen fibers around the islets of Langerhans (green arrow) and between the pancreatic acini (black arrows). **[b]** Group II demonstrating: significant collagen fiber invasion surrounding the islets of Langerhans (green arrow), between the pancreatic acini and around blood vessels (black arrow). **[c]**: Group III demonstrating: mild to moderate amount of collagen strands around blood arteries and in between pancreatic acini (black arrow) and around islets of Langerhans (green arrow). **[d]:** Group IV demonstrating: A little quantity of collagen fibers surrounding the islets of Langerhans (green arrow), between the pancreatic acini and surrounding blood vessels (black arrow). **[e]:** Group V demonstrating: minimal amount of collagen fibers surrounding the islets of Langerhans (green arrow) and between the pancreatic acini and blood vessels (black arrow). (Masson's Trichrome ×200)

**Caspase immunostain results:**

The control group's pancreatic tissue section stained with caspase immunostain revealed negative immunoreaction in the islets of Langerhans and acinar cells (Fig. 3a), Acinar cells and islets of Langerhans in group II, however, displayed significant positive immunoreactions (Fig. 3b). Acinar cells and the islets of Langerhans showed mild to moderate positive immunoreactions in the section of group III (Fig. 3c). Group IV displayed mildly positive immunoreactions in both the islets of Langerhans and acinar cells (Fig. 3d), eventually, group V displayed negative immunoreactivity in the islets of Langerhans and acinar cells that was almost identical to the control (Fig. 3e).

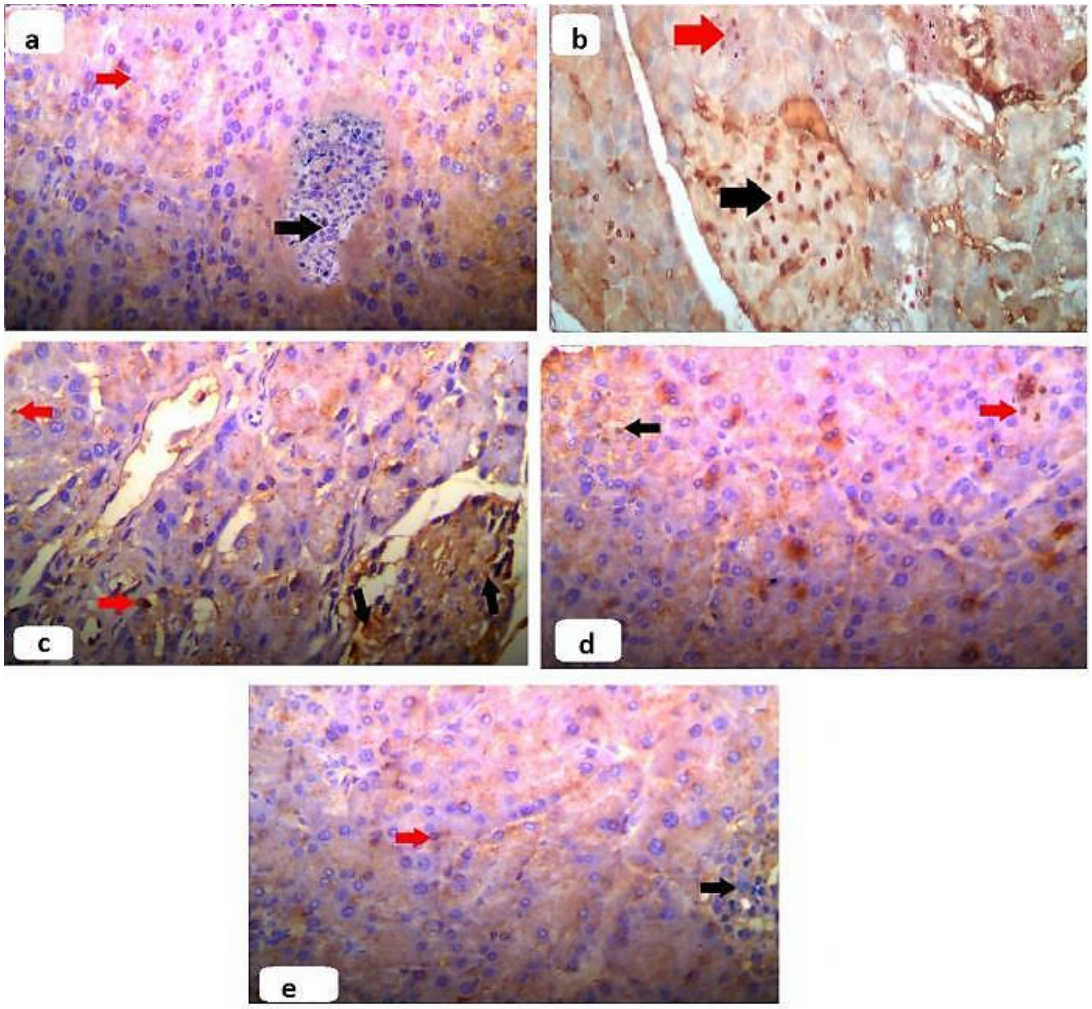


**Figure 3.** A photomicrograph of a slice of pancreatic tissue taken from albino rats showing: **[a]**: control group (group I) demonstrating: negative immunoreaction in the islets of Langerhans (black arrow) and acinar cells (red arrow). **[b]:** Group II demonstrating: Acinar cells exhibit robust positive immunoreactions. (red arrow) also in islets of Langerhans (black arrow). **[c]:** Group III showing: mild positive immunoreactions in acinar cells (red arrows) and in islets of Langerhans mild to moderate positive immunoreactions was detected (black arrows). **[d]**: Group IV demonstrating: mild positive immunoreactions in acinar cells (red arrow), and in islets of Langerhans mild positive immunoreactions was detected (black arrow). **[e]:** Group V demonstrating: negative immunoreaction in the islets of Langerhans (black arrow). and acinar cells (red arrow). (Caspase x400).

**Morphometric Results:**

Table (2) shows the mean area % of collagen fiber deposition. Collagen fiber deposition was highly notably deposited in group II in comparable to group I. Collagen fiber deposition was significantly depressed in groups III, IV, and V than in group II. Comparing group V to group III, V there was a substantial decrease in the deposition of collagen fibers.

**Table (2):** Demonstrating the average area % and SD of collagen fibers deposit in groups I, II, III, IV and V with comparison among all groups via Post Hoc LSD test

| | Group I Control (n=10) | Group II RB (n=10) | Group III Quercetin + RB (n=10) | Group IV Omega-3+ RB (n=10) | Group V Quercetin+ omega-3+ RB (n=10) |
|---|---|---|---|---|---|
| mean area % and SD of collagen fibers | 0.28%±0.0545 | 6.58% ±0.5786 a, c, d& e** | 3.96%±0.2677 b* | 3.2%±0.3377 b* | 0.29%±0.0847 b, c, and d* |

n: number, SD: standard deviation. **P**:p-value *: Significant (P≤0.05). **: Highly significant (p≤0.001).
a: Significantly highly different with group I
b: Significantly different with group II, c: Significantly different with group III
d: Significantly different with group IV, e: Significantly different with group V

Table (3) presents statistical data showing that the energy drink (group 2) had a considerably higher mean area percentage of caspase-3 immunostaining than the control animals (group 1). All rats in groups III, IV, and V had significantly lower levels of caspase-3 immunostaining than the energy drink group (group II). Group V, which got both treatments, showed a significant decrease in caspase-3 immunostaining as compared to those that received either III or VI therapy.

**Table 3.** Caspases-1 activity levels in experimental animals' pancreas

| groups | Group I Control (n=10) | Group II RB (n=10) | Group III Quercetin + RB(n=10) | Group IV Omega-3 + RB(n=10) | Group V Quercetin+ omega-3 + RB(n=10) |
|---|---|---|---|---|---|
| Pancreas (pM/mg protein *Mean ± SD* | 10.1 ±1.515 | 82.2 ± 7.866 $^{a, c ,d \& e**}$ | 33 ±1.712 $^{b*}$ | 24 ± 3.145 $^{b*}$ | 10.6 ±1.505 $^{b,c \& d*}$ |

**SD**: Standard deviation, n: number

**P**:p-value **,*:** Significant (P≤ 0.05). ****:** Highly significant (p≤0.001).

a: Significantly highly different with group I

b: Significantly different with group II

c: Significantly different with group III

d: Significantly different with group IV.

e: Significantly different with group V

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## DISCUSSION

Energy drinks are now commonly utilized by athletes and young individuals globally to enhance their physical performance, alertness, focus, and attentiveness [17]. In recent decades, a variety of studies have examined quercetin's potential as a treatment for diabetes, heart disease, renal illness, neurological diseases, cancer, and reproductive dysfunction [18].

According to **Liu *et al.*** omega-3 FA has a quick anti-inflammatory effect on the early inflammatory cascade [19].

The energy drink-treated group (group II) in our study had much lower GSH and SOD levels and significantly higher MDA levels than the control group, which is consistent with **Haroun *et al.*** **[20]**. Additionally, according to **Ayuob and ElBeshbeishy** energy drinks' effects on the pancreas have been connected to oxidative stress conditions, which manifest as decreased production of GPX and SOD[21].

According to **Hulail *et al.*** the pancreatic tissue's SOD and GSH levels decreased in the red bull-treated group [7]. According to **Sharma and Sangha** SOD transforms the extremely reactive superoxide anion into hydrogen peroxide ($H_2O_2$), neutralizing it. Therefore, it is one among the first lines of defense that shields cells from harm brought on by oxidative stress [22].

However, this study found that the pancreatic tissues of groups III and IV had a Notable decline in MDA concentrations and a substantial boost in GSH and SOD levels.

It is interesting to see that group V rats' combined use of energy drinks containing quercetin and omega 3 considerably restored all of the altered parameters to levels that were similar to group I.

Prior research indicated that quercetin was a more potent anti-inflammatory and antioxidant molecule and that ROS suppression was linked to the (3,5,7,4') locations of hydroxyl groups. The quercetin antioxidant and anti-inflammatory qualities may therefore be responsible for its therapeutic and preventive effects through a number of different mechanisms[23].

Furthermore, GSH's ability to donate hydrogen was also markedly enhanced. However, cells were able to dramatically enhance the production of SOD after receiving quercetin. **Li *et al.*** reported that while quercetin restored normal levels of GSH content and SOD activity, these parameters drastically reduced in diabetic mice [24].

**Batiha *et al.*** states that quercetin increases superoxide dismutase (SOD), glutathione (GSH), and lipid peroxidation (LPO), and decreases malondialdehyde (MDA), and it possesses antioxidant qualities [25].

Omega-3 antioxidant activity was demonstrated by a notable rise in SOD and GPX levels in pancreatic tissue, according to **Ayuob and El Beshbeishy**[21]. Additionally, **Hendrawati and Winardi** showed that by boosting antioxidant enzymes including supplementing with SOD, glutathione peroxidase, catalase, and omega-3 fatty acids, might enhance the synthesis of endogenous antioxidants [26].

According to the current experiment, group II H&E-stained sectors showed numerous degenerated acini with extensive inflammatory cell infiltration and vacuolations between acini. This is matched with the research of **Haroun *et al.***, who found that rats' pancreatic cytoarchitecture was significantly distorted upon histological examination of the organ following energy drink consumption **[20]**.

In a study by **Ayuob and El Beshbeishy,** rats given energy drinks displayed distorted architecture, varying degrees of degeneration of the acinar and islets' cells, as evidenced by intracellular taking supplements of omega-3 fatty acids, glutathione peroxidase, catalase,

and SOD and small pyknotic nuclei or even lost nuclei. These alterations result from the combination of the energy drink's various ingredients, particularly its high caffeine concentration, which creates an environment that is pro-oxidant [21].

Additionally, **El Desouky *et al.*** found that rats given energy drinks had distorted architecture in their pancreatic sections, with varying degrees of degeneration of the acinar and islets' cells manifested as reduced zymogen granules, fatty deposits, dilated engorged blood vessels, tiny pyknotic nuclei or even absent nuclei, and intracellular vacuolations [9]. **Bawazir *et al.*** [27] explained these structural alterations by pointing to the inflammatory reaction that energy drinks trigger, which leads to the emission of pro-inflammatory cytokines that lead to a number of degenerative alterations.

Acinar and islet cell cytoplasmic vacuolations seen in the energy beverage group were explained by **Khayyat *et al.*** as being caused by fatty degeneration and the buildup of degenerative elements in the cytoplasm [28].

According to some studies, mononuclear cellular infiltration occurs as an inflammatory response that removes pathologic insults and removes damaged tissue components to facilitate tissue restoration [29].

According to **Khayyat *et al.*** taurine's distinct reactivity with other energy drink active components, such as caffeine, may be the cause of blood vessel congestion and leucocyte infiltration [28].

In this study, the histological construction of the pancreas improved in groups III and IV, with the exception of a few vacuoles, but the blood vessels remained clogged. The exocrine acinar cells appeared normal with mild inflammatory cellular infiltration, while Group V displayed a reasonably normal pancreatic tissue structure. Almost normal islets of Langerhans with somewhat clogged blood arteries encircled by most likely natural exocrine architecture of pancreatic tissues acinar cells were among the notable improvements in the histological pancreatic tissue.

According to **Li *et al.*** quercetin protected the integrity of the pancreatic islets, including their area, periphery, and structure. They further demonstrated that the giving of quercetin led to an increase in insulin optical density and islet β cells [24]. **Ayuob and ElBeshbeishy** demonstrated that when omega-3 was administered in conjunction with energy drinks, the pancreatic tissue was clearly protected from the harmful effects of the drinks on rat pancreatic sections. This could be explained by omega-3's antioxidant and anti-inflammatory properties[21].

The energy drink-treated group in this study displayed extensive collagen fiber infiltration surrounding the islets of Langerhans and between the pancreatic acini and blood vessels.

Additionally, according to **Haroun *et al.*** rats given energy drinks had a markedly higher mean percentage of their pancreatic collagen fibers [20]. According to **Mubarak** the energy drink-treated group's higher area percentage of collagen fibers may be related to the harmful effects of caffeine [30].

Groups III and IV displayed mild to moderate levels of collagen fibers surrounding Langerhans' islets and in around the pancreatic acini and blood vessels, but group V displayed negligible levels of collagen fibers in these areas.

**Marcolin *et al.*** [31] reported that quercetin administration has an antifibrogenic effect on several levels, primarily through the control of profibrogenic signals produced by oxidative stress and inflammation.

In addition to promoting overall health, omega-3 plays a crucial role in reducing chronic inflammatory conditions as inflammatory bowel disease and other inflammatory gastrointestinal disorders [32].

The group that consumed energy drinks in this study displayed significant positive immunoreactions in the islets of Langerhans and acinar cells.

Comparable to the control group, the energy drink-treated group's mean color potency of the active caspase III immunohistochemistry positive reaction increased significantly, according to **Abonar *et al.*** morphometric analysis [33].

Furthermore, **Haroun *et al.*** demonstrated a considerable rise the proportion of islet cell cytoplasmic caspase-3 immuno-expression compared to pancreatic acini's cytoplasm [20]. According to **Ayuob and El Beshbeishy**, the increase in caspase-3 immunoreactivity with energy drinks was attributable to the influence of energy drinks' elevated caffeine content on oxidative stress-induced apoptosis [21]. While **Mubarak** specified that the nuclear alterations might be triggered by the beverages' preservatives, such as sodium benzoate, have the potential to produce the carcinogenic chemical benzene when combined with ascorbic acid, another customary ingredient in energy drinks [30].

Immunostained sections of groups III and IV in this investigation displayed mildly positive acinar cell and mild to moderately positive islets of Langerhans immunoreactions, but group V displayed negative immunoreactions in both islets of Langerhans and acinar cells that were almost identical to control.

**Tzifi *et al.*** [34] found that indications of cell survival were acquired following quercetin administration. The lowering of inflammatory acuteness through the mechanism of drastically lowering TNFa output, IL-6, and raising BCl2 expression demonstrated cell survival. **Pang *et al.*** [35] claim that quercetin can influence CD36 and lower the rate of pancreatic cell mortality via promoting fatty acid manufacture, promotion cell sticking, boosting the immune system, and controlling thrombospondin-1.

According to the area percent, omega-3 may specifically decrease the quantity and degree of caspase-3 immunoexpression, which indicates apoptotic cellular rescue in the pancreas [21].

Consistent with a previous study **Hendrawati and Winardi,** the present findings indicated that quercetin and omega-3 have a synergistic impact on biochemical parameters and the histological damaging effect of energy drinks on the pancreas**,** which evaluates how quercetin and omega-3 combined therapy diminishes the phrase of NFκB (nuclear factor kappa B, which damages cells in diverse organs, including the pancreas of rats with type-2 diabetes) [26]. Thus, according to our research, when quercetin and omega 3 are combined, the results are better than when either substance is used alone, and, hence, it is suggested for protection from the risks of energy drinks on the pancreas.

## CONCLUSION

Energy drinks have been observed to threaten public health leading to many medical problems. It has a negative impact on the integrity of rat pancreas. When quercetin and omega-3 were administered alone or in combination, these alterations were reversed.

## RECOMMENDATION

It is recommended to have additional investigations for how energy drinks affect other human organs.

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**Conflicts of interest:** The authors have stated that they have no conflicts of interest.

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