Original Article

To What Extent, Can Quercetin and Omega 3 Ameliorate Cardiac Muscle Changes Caused By Energy Drinks in Adult Male Albino Rats: Histological and Immunohistochemical Study

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ABSTRACT

Introduction: Athletes and youth consume energy drinks (Eds) more often. They are sold as supplements that increase both mental and physical performance. Prolonged use of these increases the risk of cardiovascular disease.

Aim of the Work: The present work aimed to ascertain the extent of cardiac injury induced by EDs in mature male albino rats and explore the possible cardioprotective impacts of Quercetin and Omega 3.

Materials and Methods: Forty adult albino rats were separated into 4 identical groups: the control group, the Omega 3 (Om) group, the Quercetin (Qu) group, and the Energy drink (ED) group. The ED group received Red Bull at a dose of 1.5 ml/100 g body weight , the Omega 3 group received both Red Bull and omega 3 at a dose of 300 mg/kg body weight ,while Quercetin group received both Red Bull and Quercetin at a dose of 75 mg//kg body weight . For four weeks, each therapy was administered by gastric gavage once a day. The goal of obtaining blood samples was to determine the level of cardiac enzymes. Heart muscle samples were taken and prepared for histological and immunohistochemical examinations.

Results: The anti-oxidant enzyme activity, MDA level, and serum cardiac enzyme level were all significantly altered in the ED treated group. The H&E stained sections revealed dark pyknotic nuclei in certain cardiomyocytes along with aberrant architecture of the cardiac muscle fibers with broad endomysium between them. P53, Desmin, and anti-fibronectin immunoreactivity were also shown to be disturbed. All of these results point to negative effects of EDs on the heart muscle structure. Treatment with Omega 3 or quercetin improves the histopathological results and biochemical measures.

Conclusion: Since quercetin and omega 3 have anti-inflammatory, anti-oxidant, and anti-apoptotic qualities, they may be able to lessen the potentially detrimental effects of energy drinks on the heart.

Received: 29 April 2024, **Accepted:** 21 June 2024

Key Words: Cardiac muscle, energy drinks, omega 3, quercetin.

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ISSN: 1110-0559, Vol. 48, No. 3

INTRODUCTION

Young people have been consuming energy drinks (EDs) more frequently, which are non-alcoholic beverages. It may be due to their ability to promote alertness, enhance mental and physical performance, and reducing fatigue. The well-known brands of energy drinks in Egypt are Power Horse and Red Bull[1].

The Red Bull energy drink contains potent stimulants, including a substantial amount of caffeine, along with various psychoactive substances such as glucuronolactone, the amino acid taurine, vitamin B complex, panthenol, niacin, and herbal sources like ginseng and guarana[2]. After Ross Coony died after consuming four cans of Red Bull before the play start, France implemented a ban on the popular energy drink, namely Red Bull. In addition, Brittan issued a warning against the use of Red Bull by pregnant women and children[3]. Energy drinks may have several detrimental consequences on the body, such as high blood pressure, irritation, lack of energy, distorted thinking, difficulty concentrating, and difficulties with cognitive function. Additionally, they can also have harmful effects on the liver and kidneys[1]. The mechanism of Red Bull generated detrimental effects has been associated with oxidative stress. Therefore, the use of antioxidants may be beneficial in mitigating these effects[4].

Several plants have been shown to possess extensive cardioprotective effects[5]. Quercetin (Qu) is a natural flavonoid that is often present in several vegetables and fruits, including red onion, apple, citrus fruits, berries, tea, nuts, and seeds[6]. Quercetin has garnered significant interest due to its antioxidant and anti-inflammatory characteristics[7]. Research has shown that the addition of Quercetin enhances the functioning of antioxidant enzymes, including catalase activity (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx)[8]. Subsequent research has shown a clear relationship between polyunsaturated fatty acids consumption and a reduced likelihood of developing cardiovascular disorders, including as heart failure, hypertension, arrhythmias, and coronary heart disease[9]. The omega-3 polyunsaturated fatty acids consist of eicosapentaenoic acid and docosahexaenoic acid[10]. The primary source of

DOI: 10.21608/ejh.2024.283932.2059

these fatty acids is sea food, however a minor quantity is produced inside the body utilizing linolenic acid. The fatty acids are found in plant sources such as canola, flaxseed, and walnut[11]. Omega 3 has anti-inflammatory and antioxidant characteristics[12]. According to Hooijmans *et al*.[13], extended usage of a treatment had positive effects on factors associated with animal models of Alzheimer's disorder.

The purpose of this study was to examine the impact of Red Bull on the heart muscle of adult albino rats and to investigate the cardioprotective effects of Quercetin and Omega 3 against Red bull induced cardiac damage. The effects were evaluated using serological, histological, immunohistochemical, and morphometric examinations.

MATERIALS AND METHODS

Animals

The investigation was implemented on a sample of forty male adult albino rats that were deemed to be in a good health. The rats had an average weight ranging from 180g to 220g. Animals were acquired from the laboratory animal unit at the Faculty of Veterinary Medicine, Zagazig University, Egypt. In controlled laboratory settings at anatomy department, Faculty of Medicine, Benha University, rats were kept in separate plastic cages and given with enough water and food. The animal care ethical criteria were over seen by the Faculty of Medicine, Benha University, with an approval number of Rc 27-9-2023.

Chemicals

Red Bull energy drink: A 250 mL can was purchased from a nearby store in Benha, Qalyubia, Egypt.

Quercetin: was in the form of solid substance, purity 95%, with a CAS number of 117-39-5 (Sigma-Aldrich Chemie GmbH). Distilled water was used to dissolve it[14].

Omega-3: 300 soft gelatin capsules were acquired from Wael Samir's pharmacy and made by PHARCO Company Pharmaceuticals in Cairo, Egypt. The capsules consist of 1000 mg of fish oil, which contains about 30% omega 3. The fish oil was extracted using a syringe and administered to the rats orally by gavage[15].

Experimental design

Following a period of one week for adaption. The rats were placed into four groups, each consisting of ten rats.

Group I (control group) is split into three subgroups:

- Ia: four rats received distilled water and regular diet for 4 weeks.
- Ib: three rats received Quercetin once daily for 4 weeks via oral gavage (75 mg/kg/day)[16].
- Ic: the last three rats was given 300 mg/kg of omega 3 which corresponding to 0.02 ml fish oil/ rat once daily by oral gavage for 4 weeks[15].

Group II (ED group): Red Bull was given to rats at a dose of 1.5 ml/100 g b.w. by oral gavage once daily for 4 weeks[15].

Group III (Om group): Rats were received both Red Bull as group II, and omega 3 at a dose of 300 mg/kg body weight once daily through oral gavage for 4 weeks.

Group VI (Qu group): Rats were received both Red Bull as group II, and Quercetin at a dose of 75 mg/kg body weight once daily through oral gavage for 4 weeks.

All rats were given ether inhalation anesthesia after the allotted time for each group. For the biochemical analysis, blood samples were taken straight from IVC and put in tubes that were non-heparinized. Centrifugation of blood samples were done for 15 minutes at 3000 rpm, and the serum was taken out and stored at -20° C.

Each heart was taken away and then separated into two halves along its length. Half of the sample was promptly stored at a temperature of -80 °C for further homogenization, which would be used to assess antioxidant activity. The other half of cardiac tissue was submerged in 10% neutral-buffered formalin for the purpose of conducting histological and immunohistochemical examinations.

Biochemical Parameters

The blood samples assessed the cardiac enzymes level, Creatine kinase (CK) and lactate dehydrogenase (LDH). The measurement was conducted using kits provided by Sigma Aldrich Co.[17].

Cardiac antioxidant activity and lipid peroxidation are being studied. Heart tissues were homogenized using PBS with a pH of 7.4. Centrifugation was carried out and the resulting supernatant was collected for the purpose of determining enzymatic activity. In order to assess the extent of oxidative damage in the heart, the concentration of MDA (a biomarker for lipid peroxidation), SOD activity and CAT activity were quantified using a commercially available ELISA kit (ABclonal ELISA Kit Catalog No. RK00691, RK03959, RK01041) respectively following the instructions provided by the manufacturer.

Light microscopic examination

The All rats' left ventricles were quickly sampled for cardiac tissue and were fixed in formalin, after that dehydrated using increasing concentrations of ethanol and then using xylene. Subsequently, the paraffin blocks were done and 5 µm-thick slices were cut for further analysis.

1-Histological study:

Hematoxylin and eosin (H&E) stain was used for regular histological investigation. Additionally, Masson’s trichrome stain was used to determine the quantity of collagen present between the heart muscle fibers[18].

2- Immunohistochemical staining:

a. Anti-Fibronectin antibody: mouse monoclonal antibody (abcam, EPR23110-46).

b. Tumor protein P53: a mouse monoclonal anti-P53 (abcam, PAb 240) primary antibody.

c. Desmin protein: Desmin monoclonal Antibody (abcam, SP 138).

The immunoperoxidase system streptavidin–biotin complex was employed. After deparaffinizing serial sections embedded in paraffin on positively charged slides, the sections were incubated for 30 minutes at room temperature with the matching primary antibody, followed by 30 minutes in 0.1% hydrogen peroxide to inhibit endogenous peroxidase. Following many PBS washes, the sections were ready for the secondary antibody incubation. After incubation with the chromogen Diamiobenzidine (DAB), staining was finished. As a counterstain, Mayer's hematoxylin was employed[19].

The H&E, Masson's trichrome stained sections, and immunostained sections were analyzed by a light microscope (Olympus CX 41, Japan) and captured with a digital camera connected to the microscope at the Anatomy & Embryology department, Faculty of Medicine, Benha University, Egypt.

Histomorphometric analysis

The area percentage of collagen stained with Masson's trichome and the average area percentage of antifibronectin, P53, and Desmin positive reactivity in sections were assessed in fields from each group (×400). The area percentages were quantitatively evaluated using the Image J software analyzer computer. Subsequently, the collected data were expressed as the mean value and the standard deviation and subjected to statistical analysis.

Statistical analysis

The data was organized into a table, assigned codes, and then analyzed using SPSS, version 19. The information was displayed as the standard deviation (SD) minus or plus the mean value. The researchers used a one-way ANOVA test to ascertain the statistical significance between the various groups[20]. The findings were estimated statistically significant when their *P values* less than 0.05.

RESULTS

Changes in some metabolic markers and cardiac markers

ED treated rats had significantly higher levels of CK and LDH blood levels comparing with rats in all other groups ($P \leq 0.05$). However, rats given ED and treated with omega 3 or Quercetin had a significant reduction in the serum levels of CK as well as LDH when compared to ED group ($P \leq 0.05$) and these levels did not exhibit important difference from the control rats (Table 1, Figure 1a).

The level of lipid peroxidation was elevated in ED group evidenced by significant increase in the MDA level of the cardiac tissue compared to the other groups ($P \leq 0.05$), while the antioxidant enzymes (CAT and SOD) levels were significantly decreased in this group in comparison with all other groups ($P \leq 0.05$). On the other hand, omega 3 and Quercetin treated groups presented significant diminution in the MDA level and significant elevation of the antioxidant enzymes activity compared to ED group ($P \leq 0.05$) (Table 1, Figure 1b).

Hematoxylin and Eosin results

Under light microscopy, the longitudinal sliced myocardium of the control subgroups showed the typical histological structure when the cardiac tissues were stained with Hematoxylin and Eosin. Myofibrils running parallel to the longitudinal axis of the muscle fibers make up the regularly ordered cardiac fibers that make up the myocardium. The cardiomyocytes have centrally positioned oval vesicular nuclei and a cylindrical appearance with acidophilic sarcoplasm. Myocardial blood capillaries and flat-nucleated fibroblasts were separated from cardiac myocytes by a thin layer of connective tissue called the endomysium (Figure 2a).

In the ED group, the cardiac tissue displayed abnormal architecture included wavy cardiac muscle fibers. There was a wide endomysium between the cardiac muscle fibers. Some cardiomyocytes had dark pyknotic nuclei. There were others with perinuclear cytoplasmic vacuoles. Additionally, there was noticeable cardiac blood vessel dilatation and congestion and an infiltration of inflammatory cells (Figure 2b).

In Qu treated group, there was some preservation of the normal architecture of the cardiac muscle. Heart muscle fibers were arranged systematically, possessing central vesicular nuclei and few cytoplasmic vacuoles. The muscle fibers were shown to be widely separated. Some cardiomyocytes had nuclei that were pigmented darkly (Figure 2c).

In omega 3 treated group; It revealed a heart with a regular configuration, with oval fibroblast nuclei in between. Myocytes showed central oval nuclei. Mild congested blood vessel between the muscle fibers was seen (Figure 2d).

Masson's Trichrome results

Little collagen fibers were visible between the heart muscle fibers in the endomysium of control group when light microscopy was used to examine Masson's trichrome-stained slices. Increased collagen deposition was seen in the heart muscle's interrupted fiber locations in ED group. A little amount of collagen fiber formation was seen between the fibers of the cardiac muscle in the Qu and Om treated groups (Figures 3a-d).

Fibronectin results

Anti fibronectin antibody is a marker for fibronectin deposition in myocardial cells and extracellular matrix (ECM). Sections from control rats exhibited negative immunoreaction for fibronectin antibody. In Red Bull group, there was positive immunoreactivity in the form of brown deposition in the sarcoplasm of many myocardial

cells and focal areas of extracellular matrix. Omega 3 and Quercetin-treated groups displayed a very weak immunoreactivity in few myocardial cells and limited areas of extracellular matrix (Figures 4a-d).

P53 immunostained results

P53 immuno-expression in the cardiomyocyte nuclei was shown to be negative in the control groups. Positive P53 immuno-expression was seen in the cardiomyocyte nuclei of the Red Bull group. In the cardiomyocyte nuclei, Qu and Om treated groups exhibited a mild positive P53 immunological response (Figures 5a-d).

Desmin results

Desmin immunostaining revealed a pronounced brown color in the intercalated discs and Z lines of the cardiac muscle fibers of the control rats. The rats of ED group demonstrated a clear decrease in the immunoreactivity of cardiomyocyte desmin with a little brown response in intercalated discs and Z lines. Desmin's immunoreaction intensity increased in longitudinal sections of the left ventricles of Qu and Om groups, suggesting that desmin had recovered to a level that was roughly comparable to the control (Figures 6a-d).

Morphometric results

In Energy drink group, the mean area % of collagen fiber, anti-fibronectin & P53 immunoreactivity showed a statistically significant rise in comparison with other groups. While, a statistically significant decrease was detected in Quercetin treated group and omega 3 treated group when compared to ED group. Moreover, the mean area of Desmin immunoreactivity revealed a significant decrease in ED group compared to all other groups however, it was significantly increased in both omega 3 treated group and Quercetin treated group. Furthermore, there was only statistically significant distinction between the group receiving omega 3 treatment and the control group in the area of Desmin (Table 2, Figure 7).

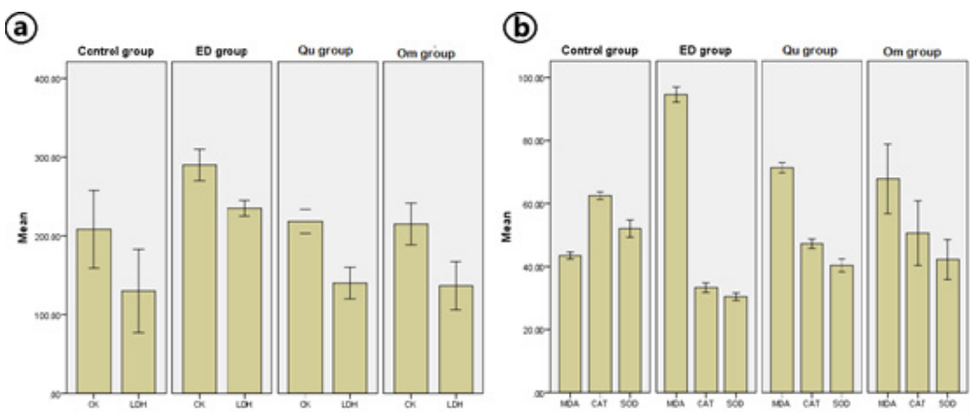

Fig 1: Data are expressed as Mean values ± SD of a) CK and LDH levels b) MDA, CAT, SOD levels in control and experimental groups: (n = 10) and analyzed using one-way ANOVA.

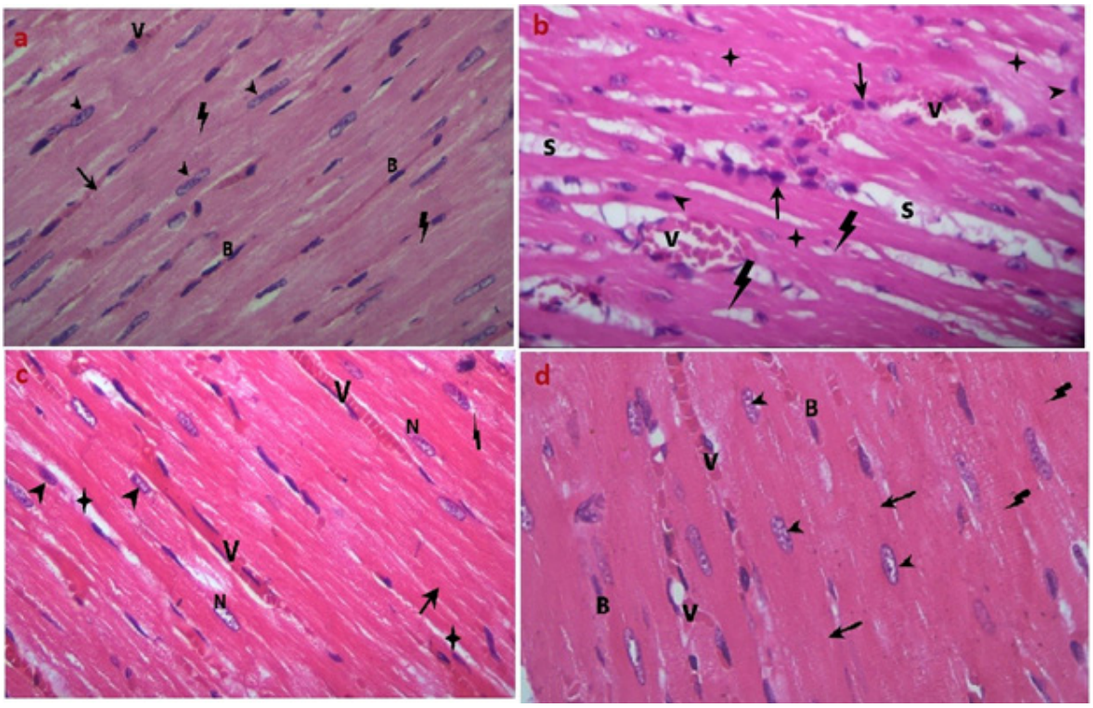

Fig. 2: Photomicrographs of longitudinal sections of the left ventricles of all experimental groups (H&E, X400): a) The control groups show myocardium consisting of consistently distributed cardiac fibers with myofibrils parallel to the muscle fibers' longitudinal axis (arrow). It exhibits acidophilic sarcoplasm with transverse striations (zigzag arrow), central vesicular oval nuclei (head arrow). The cardiac myocytes are separated by thin connective tissue which are composed of fibroblasts with flat nuclei (B) and blood capillaries (V). b) ED group shows disturbance of cardiac muscle architecture (star) with wide spaces (S) between cardiac muscle fibers. Some fibers show darkly stained pyknotic nuclei (head arrow). Markedly dilated congested blood vessel (V) with hemorrhage between fibers and inflammatory cells (arrow) are also seen. cytoplasmic vacuoles are seen (zigzag arrow). C) Qu group reveals partial preserved muscle fibers (arrow) with wide spaces (Star) and congested blood vessels (v) are still present. Myocytes show central oval nuclei (N) while some of them show pyknotic nuclei (head arrow) and few cytoplasmic vacuoles (zigzag arrow). D) Om group shows many well-organized muscle fibers (arrow). Myocytes show cross striations (zigzag arrow) in acidophilic cytoplasm and central oval nuclei (head arrow) and fibroblasts with flat nuclei (B). Notice congested blood vessel in between (v).

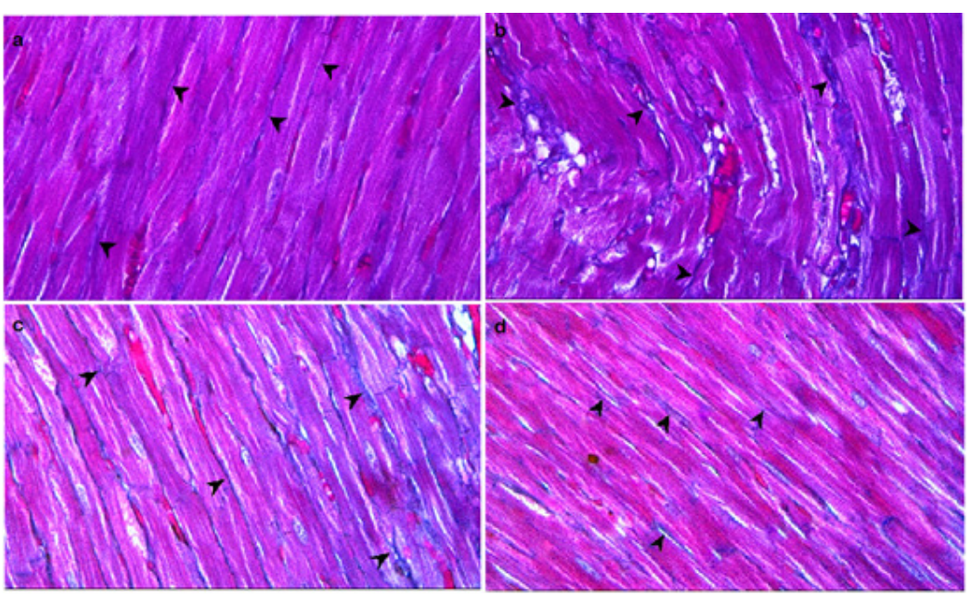

Fig. 3: Photomicrographs showing collagen deposition in longitudinal sections for the left ventricles of all experimental groups using the Masson's trichrome stain (X400): (a); for Control; (c) for Qu treated and (d); for Om treated groups; showing fine collagen fibers (head arrows) in between fibers. (b); for ED treated group displayed obvious collagenous fiber deposition (head arrows) in the locations where the heart muscle's interrupted fibers were found.

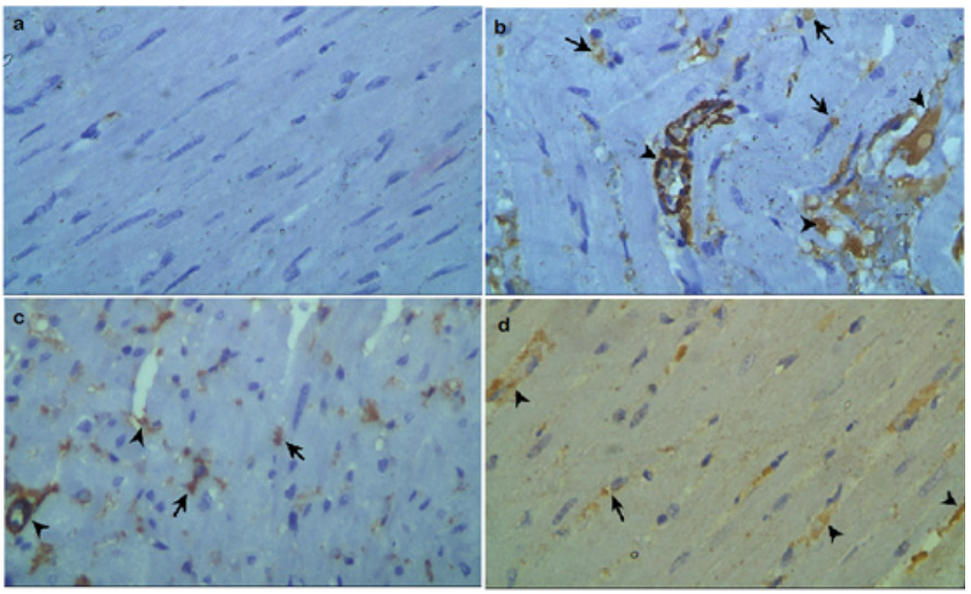

Fig. 4: Photomicrographs of longitudinal sections in the left ventricle (Fibronectin immunostaining, X400) a: The control groups show negative immunoreactivity. b: ED group exhibits positive immunoreactivity in the sarcoplasm of many cardiomyocytes (arrow) and focal areas in the connective tissue (head arrow). c: Qu group shows mild positive immunoreactivity in both cardiomyocytes (arrows) and connective tissue (head arrows). d: Om group shows faint immunoreactivity limited areas of cardiomyocytes (arrow) and connective tissue (head arrow).

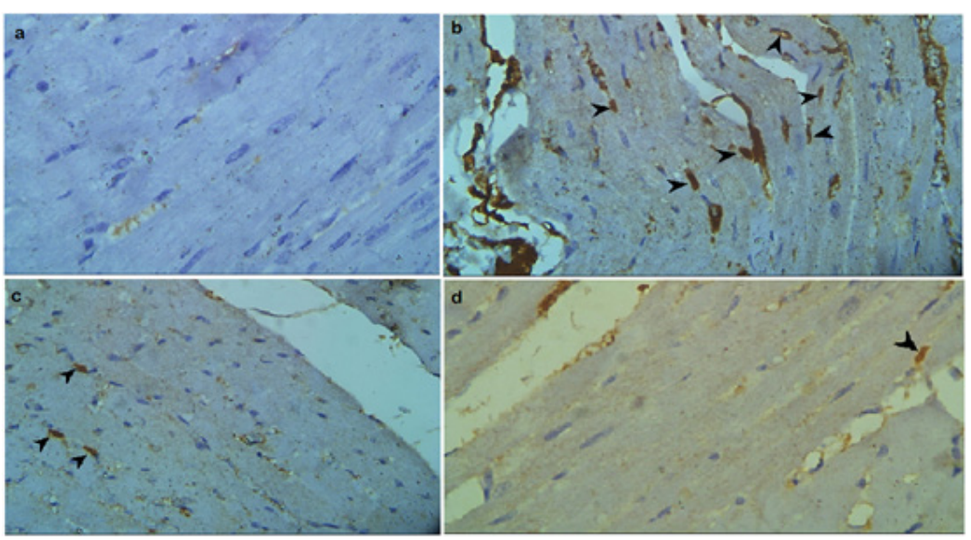

Fig. 5: Photomicrographs of longitudinal sections of the left ventricles of all experimental groups for Immuno-histochemical expression of P53 (x400) showing: (a); control groups demonstrating a negative P53 immunological response in the cardiomyocyte nuclei. (b); ED group: demonstrating a positive P53 immunological response in the cardiomyocyte nuclei (head arrows). (c); for the Qu-treated group: demonstrating a mild positive P53 immunological response in the cardiomyocyte nuclei (head arrows). (d); for the Om-treated group: a few positive P53 immunological reactions are seen in the cardiomyocyte nuclei (head arrows).

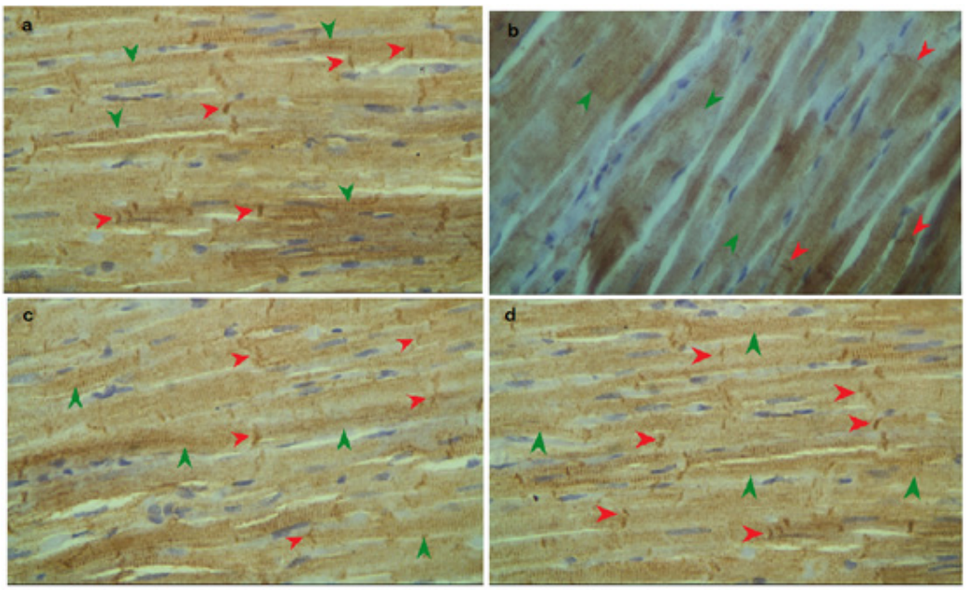

Fig. 6: Photomicrographs of longitudinal sections of the left ventricles of all experimental groups for Immuno-histochemical expression of Desmin (400×): (a) for the control group: exhibiting positive desmin expression in the Z lines (green head arrows) and intercalated discs (red head arrows) (b); ED group: exhibiting a reduction in desmin immunoreactivity, as seen by weak brown reactions in Z lines (green head arrows) and intercalated discs (red head arrows). (d); for the Om treated group, there is a clear recovery of the positive reaction to desmin in the intercalated discs (red head arrows) and Z lines (green head arrows). (c); for the Qu treated group, there is a noticeable increase in the positive reaction to desmin in intercalated discs (red head arrows) and Z lines (green head arrows).

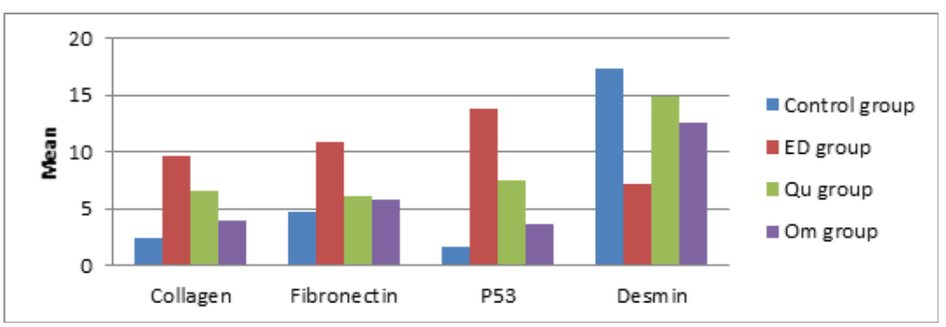

Fig. 7: Data are expressed as Mean values ± SD of of collagen fibers, area % of fibronectin, area % of P53 & area % of Desmin in control and experimental groups: (n = 10) and analyzed using one-way ANOVA

Table 1: Data are expressed as Mean values ± SD of MDA, CAT, SOD, CK and LDH levels in control and experimental groups: (n = 10) and analyzed using one-way ANOVA. Significant (P< 0.05) compared with other groups

	Control group		ED group		Qu group		Om group		ANOVA	*P value*
	Mean	±sd	Mean	±sd	Mean	±sd	Mean	±sd		
CK (U/L)	175.0 [b]	5.0	290.0 [acd]	10.0	218.33 [b]	7.64	215.0 [b]	13.23	18.68	0.000
LDH (U/L)	101.67 [b]	7.64	235.0 [acd]	5.0	140.0 [b]	10.0	136.67 [b]	15.28	28.23	0.000
MDA (nmol/g tissue)	43.47 [bcd]	0.55	94.6 [acd]	1.2	71.37 [ab]	0.81	67.83 [ab]	5.51	160.57	0.000
CAT (mmol/min/g tissue)	62.50 [bcd]	0.60	33.3 [acd]	0.75	47.27 [ab]	0.75	50.60 [ab]	5.12	62.47	0.000
SOD (u/mg protein)	52.03 [bcd]	1.36	30.43 [acd]	0.60	40.37 [ab]	1.03	42.23 [ab]	3.16	70.77	0.000

Values are considered statistically significant
a significant compared to control group
b significant compared to ED group
c significant compared to Qu group
d significant compared to group Om

Table 2: Data are expressed as Mean values ± SD of of collagen fibers, area % of fibronectin, area % of P53 & area % of Desmin in control and experimental groups: (n = 10) and analyzed using one-way ANOVA. Significant (P< 0.05) compared with other groups

	Control group		ED group		Qu group		Omgroup		ANOVA	*P value*
	Mean	±sd	Mean	±sd	Mean	±sd	Mean	±sd		
Collagen	2.43 ^{bc}	0.31	9.70 ^{acd}	0.40	6.57^{ab}	0.40	4.0 ^{b}	1.32	49.13	0.000
Fibronectin	4.70 ^{bc}	0.80	10.83 ^{acd}	0.95	6.17 ^{ab}	0.31	5.87 ^{b}	0.65	42.76	0.000
P53	1.70 ^{bc}	0.20	13.83 ^{acd}	0.31	7.53 ^{ab}	0.55	3.57 ^{b}	1.21	157.03	0.000
Desmin	17.37 ^{bd}	1.92	7.17 ^{acd}	1.25	14.93 ^{b}	1.72	12.50 ^{ab}	1.60	21.22	0.000

Values are considered statistically significant
a significant compared to control group
b significant compared to ED group
c significant compared to Qu group
d significant compared to group Om

DISCUSSION

Energy drink use has increased during the past few years. The appealing marketing tactics used by the corporations producing these beverages are one of the good reasons. Teenagers and anyone who engage in physical activity are the target market for energy drinks. The dangerously large number of these drinks that these young folks are ingesting[21].

A comprehensive research project was conducted to reveal the effects of energy drinks on male albino rats' heart muscles and the cardioprotective properties of omega-3 and quercetin.

In the present study, oral management of the rats with Red Bull produced oxidative stress state that denoted by a significant raise of the MDA level in the cardiac tissue with a significant reduction of the antioxidant enzymes activity. These results were in agreement with Abdelwahab *et al*.[15], who reported that 4 weeks administration of the two energy drinks; power horse and red bull to the adult rats prompted a significant raise of MDA serum level while, the serum SOD and CAT activities were significantly reduced. Seifert *et al*.[22] elucidated that such effects might be triggered by the combination of several ingredients in the ED and the synergistic interaction between them augmenting the oxidative impact. While Alsunni[23] proposed that caffeine is the main ingredient that causing major toxicity. Additional researchers discovered that decreased tissue sensitivity for insulin and impaired metabolism of glucose may be involved in ED-induced oxidative stress[24].

The majority of body tissues contain the LDH enzyme which elevated in response to cell injury. Its high level is a biomarker for a number of diseases, including CVDs[25]. CK is an additional biomarker that rises in response to stress or damage to the heart muscle[26]. We found that ED treated group showed a significant rise in CK and LDH serum levels compared to rats in all other groups.

Our light microscopic results showed enlarged endomysium between myofibers and confirmed earlier findings in the red bull treatment group. Spots of lacerated and wavy myofibers were present. Pyknotic nuclei and vacuum-separated cytoplasm were observed. The efficacy of the new model is confirmed by these histological alterations, which align with the findings of earlier researchers. Previous study showed several infiltrations of inflammatory cells and numerous damages to the heart muscle cells[27]. Another study examined energy drinks and alcohol. They saw huge mitochondria with dilated crystals and unusual gaps between myofibrils, which were assumed to be the result of oxidative damage[28]. Three groups of rats given energy drinks at three different dosages were compared with the control group[29]. Their examination with histopathology. Upon a histopathological study of the liver and kidney, they discovered intercellular tissue inflammation as well as cell congestion and necrosis. Costa-Valle *et al*,[30] observed vascular congestion and engorgement in the kidneys of rats given an energy drink plus alcohol for a month.

Cardiac muscle fibrosis in this study was confirmed by Masson Trichrome and fibronectin immunostaining results which exhibited a significant increase in mean area of collagen fibers and fibronectin after Red Bull administration in comparison with Control group. This was consistent with another study that explained these results by lipid peroxidation which triggers an inflammatory response with overproduction of fibrogenic cytokines, which induce fibrosis[31]. Moreover, there is a work added that, macrophages may be responsible for increasing fibronectin and platelet derived growth factor both of which motivate fibroblasts division[32].

The immunohistochemistry results of this work, which showed a notable increase in the expressions of the apoptotic protein p53 in the Red Bull group relative to the control group, corroborate the findings of H and E. Many genes involved in DNA repair and apoptosis have varying rates of transcription, which are controlled by the transcription factor p53 (tumor suppressor protein)[33]. The high nicotinamide content of energy drinks may cause apoptosis on its own by blocking SIRT1, a p53 modulator and down regulator of apoptosis, which might explain the rise in P53 immunoreactivity[34]. This was consistent with findings from a related research[35,36], which showed that giving rats energy drinks like Red Bull and Power Horse caused a significant cytoplasmic caspase-3 response in the stomach and pancreas.

When comparing the ED group to the control groups in the current study, a faint reaction to the desmin protein was seen, and the optical density of the anti-desmin antibody reaction statistically indicated a very significant difference. Our findings so concur with the previously established conclusions. The most significant intermediate filament protein in the heart is desmin, which along with other binding proteins creates a continuous network that encircles the z lines and stretches the whole width of the cardiomyocyte. It preserves the integrity of the cell and connects the contractile apparatus to the majority of membrane organelles as well as the intercalated discs[37]. Avery, (2011) has recorded that the structural integrity of cardiomyocytes were disrupted, resulting in oxidative stress-induced cellular damage. The oxidative stress is caused damage to the three main types of macromolecules—lipids, nucleic acids, and proteins[38].

In the current study, quercetin treated group presented a significant decrease in the tissue MDA level and a significant elevation of CAT and SOD enzymes activity compared to ED group. This was in accord with a research documented that bisphenol-A raised MDA level and reactive oxygen species (ROS) in the cardiac mitochondria inducing oxidative stress, but treatment with quercetin limited this effect and significantly elevated CAT activity[8]. The structural reasons for quercetin's antioxidant abilities include the presence of hydroxyl group at position C3 and a carbonyl group at C4, which enable the compound to chelate with iron ions and counteract free radicals[39].

our results presented a significant reduction in the serum levels of CK and LDH in rats treated with quercetin compared to ED group. Similarly, previous study reported that administration of each quercetin and Sitagliptin in doxorubicin treated rats was effective in restoring serum CPK, LDH and troponin to the standard levels[40]. In addition, it was established that quercetin could improve LDH and CK enzymes activity[8].

In this work, it demonstrated that the Quercetin treated group had a normal structure of the myocardium with wide space and cytoplasmic vacuolization. By using Mallory's trichrome stain and fibronectin immunostaining after quercetin administration, we observed reduced collagen fiber deposition in interstitial tissues. According to earlier research, Quercetin was beneficial for cardiomyocytes with normal parallel myofibrils and sarcomeres; however, in certain regions, the myofibrils were thin and separated, and it also suggested that there was cardiac fibrosis[21]. According to earlier clinical research, Quercetin shows strong heart morphological improvements and cardioprotective benefits[41].

The present study showed that Quercetin treatment was able to prevent increase in p53 expression; revealed its anti-apoptotic activity. A significant reduction in p53 expression indicated that quercetin exhibited anti-apoptotic properties[14]. In the heart sections of diabetic rats, quercetin reduced oxidative stress, inflammation, and apoptosis, according to a different published study[42].

Strong positivity to the anti-desmin antibody demonstrated desmin protein recovery, which is in line with results from electron microscope analysis of heart sections from rats given Quercetin following high-fat diet-induced cardiac muscle damage. It revealed that each sarcomere stretched between two Z lines that followed, with dark and light bands switching places. Intercalated discs were used to link the nearby cardiomyocytes[21].

In the current study, omega 3 treated group presented a significant decrease in the MDA level with significant elevation of the antioxidant enzymes activity compared to ED group. A previous study outlined that the anti-oxidant properties of omega-3 are due to free radicals scavenging ability and reduction of lipid peroxidation[43]. Two potential mechanisms exist for omega-3fatty acids to mitigate oxidative damage; raise the amount of catalase in the cytoplasm to guard against free radicals and they might be deputize for polyunsaturated fatty acid membrane components that were released by oxygen-free radicals like hydrogen peroxide[44].

Our results established that rats received ED and treated with omega 3 displayed a significant reduction in the serum levels of CK and LDH and these levels did not differ significantly from the control rats. This was in line with a study on rats injected with doxorubicin then given 400 mg/kg omega 3 fatty acids daily for a month; it had noticeably lower blood concentrations of CK than the control group[11].

The current work showed that the group receiving omega 3 treatment had a myocardial improvement with a normal shape. We observed a decrease in the collagen deposition in interstitial tissues. The anti-inflammatory and antioxidant properties of omega 3, which prevent lipid peroxidation and eliminate free radicals, may be the cause of these alterations. The data of the current study is agreed with the result of an earlier research that revealed an improvement in ECG, cardiac function biomarkers and histopathological findings by omega 3 in doxorubicin-treated rats[45]. Leukocyte activity and the cell-mediated immune response were suppressed in order for omega 3 to have its anti-inflammatory effects[46]. It has previously been established that omega-3 has antifibrotic effects in cardiac tissues, down regulating profibrogenic genes or altering the cell membrane composition to reduce inflammation and fibrosis[10,47].

Moreover, the present study showed that omega 3 treatment was able to prevent increase in p53 expression; revealed its anti-apoptotic activity. That was in agree with many studies showing apoptotic biomarkers improvement in the cardiac tissues of gentamicin or doxorubicin induced cardiac degeneration rats when pretreated with omega 3[10,45].

A high positivity reaction to the anti-desmin antibody suggested that the desmin protein had recovered. This was statistically demonstrated by the fact that there was a significant variance between the groups receiving omega 3

treatment and energy drinks, with a significant difference to the control groups. This implies that omega 3 may offer defense against the harmful effects of energy drinks on the heart muscle. According to previous study, omega 3 has a beneficial impact on the ventricular myocardium as seen by the persistence of Z- and H-lines in the heart by electron microscopy[10].

CONCLUSION

Energy drinks might be the reason for the histological and immunohistochemical changes in rats' heart muscles. Quercetin and omega 3 may be alleviate the potentially harmful effects of energy drinks on the heart through their anti-inflammatory, anti-oxidant, and anti-apoptotic properties.

CONFLICT OF INTERESTS

There are no conflicts of interest

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الملخص العربى

إلى أي مدى يمكن للكيرسيتين والأوميجا ٣ تحسين التغيرات في عضلة القلب الناتجة عن مشروبات الطاقة في ذكور الجرذان البيضاء البالغة: دراسة نسيجية وكيميائية مناعية

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المقدمه: يستهلك الشباب والرياضيون مشروبات الطاقة بشكل متكرر. حيث يتم تسويقها كمكملات لتحسين الطاقة والتركيز والأداء المعرفي والبدني.و قد يؤدي استهلاكها لفترات طويلة إلى مخاطر صحية خطيرة تتعلق بأمراض القلب والأوعية الدموية.

الهدف: تهدف الدراسة الحالية إلى معرفة التأثير السلبي لمشروبات الطاقه على عضلة القلب في ذكور الجرذان البيضاء البالغة و التحقق من التأثيرات الوقائية المحتملة للكيرسيتين وأوميجا ٣.

المواد والطرق المستخدمة: تم تقسيم ٤٠ من ذكور الجرذان البيضاء الى أربع مجموعات متساوية : المجموعة الضابطه, مجموعة مشروبات الطاقة (ريد بول) (١,٥ مل/١٠٠ جرام /يوم), مجموعة أوميجا ٣ (تناولت كلا من ريد بول ، وأوميجا ٣ بجرعة ٣٠٠ مجم/كجم/يوم), مجموعة كيرسيتين (تناولت كلا من ريد بول ، وكيرسيتين بجرعة ٧٥ مجم/كجم/يوم).تم إعطاء العلاج لجميع المجموعات عن طريق أنبوب تغذية المعدة مرة واحدة يوميا لمدة ٤ أسابيع. تم سحب عينات الدم لقياس مستوى إنزيمات القلب. كما تم جمع عينات من عضلة القلب وتحضيرها لإجراء التحاليل البيوكيميائية والنسيجية والمناعية.

النتائج : أظهرت المجموعة المعالجة بمشروبات الطاقه تغيراً ملحوظاً في مستوى إنزيمات القلب و كذلك الإنزيمات المضادة للأكسدة. وقد تم ملاحظة تغيير فى بنية ألياف عضلة القلب مع وجود بطانة واسعة بينها بالاضافه إلى وجود أنوية داكنة في بعض خلايا عضلة القلب في نتائج الهيماتوكسيلين والايوسين. و قد أظهرت الفحوصات الكيميائيه المناعيه اضطراب فى التعبير المناعى للفيبرونكتين و P٥٣ و Desmin .

تشير هذه النتائج معًا إلى أن مشروبات الطاقة كان لها آثارا ضارة على عضلة القلب. أدى استخدام الكيرسيتين أو أوميجا ٣ إلى تحسين المعايير البيوكيميائية والتغيرات النسيجية.

الخلاصة: قد يكون كلا من الكيرسيتين وأوميجا ٣ قادرين على التخفيف من الآثار الضارة المحتملة لمشروبات الطاقة على عضلة القلب من خلال خصائصهما المضادة للالتهابات والأكسدة وكذلك المضادة لموت الخلايا المبرمج.