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Ameliorative Effect of Omega-3 on Energy Drinks - Induced Pancreatic Toxicity in Adult Male Albino Rats

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Abstract: The world wide increasing popularity of the energy drinks and the lack of information about their possible hazardous effects on health is a matter of controversy and research. The aim of this study is to assess the histological and histochemical effects of energy drinks on the pancreas of adult male albino rats and the possible protective effect of omega-3.

Keywords: Energy drink, insulin resistance, islets of langerhans, omega-3, pancreas.

Introduction

Energy drinks are non-alcoholic, lightly carbonated beverages that are designed to give the consumer a dose of energy. Public consumption of energy drinks has greatly increased over the past decade with the majority of users being adolescents and adults less than 35 years of age [1]. There are different types of energy drinks, with names like Boom Boom, Power horse, Burn, Monster, Red Bull and AMP Energy. Energy drinks have sugar-containing and sugar-free versions. Red Bull is the most popular energy drink consumed in Egypt [2]. Energy drinks mostly contain caffeine, other plant based stimulants (guarana, ephedrine, yerba mate), sugars and their derivatives (glucose, fructose, sucrose, ribose and glucuronolactone; which is a naturally occurring glucose metabolite), amino acids (taurine, carnitine, creatine), other herbal extracts (ginseng, ginkgo biloba), maltodextrin, inositol, vitamin B complex and other ingredients [3]. According to the manufacturers, the stimulating effects of these drinks are due to interaction between the various ingredients. They claim that these drinks improve physical endurance, reaction speed and concentration [4]. There are several studies recording a modest improvement of energy drinks consumption on physical endurance [5,6,7], but also studies that showed no significant enhancement of endurance related to the consecutive energy drinks consumption[8]. Some of the compounds found in energy drinks are used in therapy for treating certain disorders, like taurine and niacin for dyslipidemias. Other compounds, like glucuronolactone, are not well studied. An alarming number of side effects and even deaths were reported, as a consequence of energy drinks consumption. Arrhythmia, cardiac arrest and hepatitis are some of the quoted side effects [9,10,11]. Despite the popularity of these drinks, their effects on consumers' health are quite controversial and not sufficient research on energy drinks' safety has been conducted yet. Omega-3 in Fish oil is one of the most important polyunsaturated fatty acids (PUFA) that have an antiinflammatory and an antioxidant activity [12]. It is a blend of two essential fatty acids: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)[13]. Also, recent studies 325 El Desouky et al. suggested that dietary intake of Omega-3 could be useful in prevention of diabetes; as it reduced the activity of the pro-inflammatory processes which stimulated the body to attack its own insulin producing cell [14]. With energy drinks becoming a worldwide phenomenon, the short- and long-term effects of these beverages must be evaluated more closely in order to fully comprehend their impact on different body organs [15]. The aim of this study was to assess the effects of Red Bull as one of the most popular energy drinks on the pancreas of adult male albino rats and the possible protective effect of omega-3

Material and Methods

Drugs Energy drink is available in the Egyptian market in the form of cans 250 ml. Each 100 ml containing: a mixture of water, sucrose, glucose, sodium citrate, carbon dioxide, taurine (0.4%), caffeine (0.03%), gluconolactone (0.24%), inositol, niacin (8 mg), pantothenic acid (2 mg), vitamin B6 (2 mg), B12 (0.002 mg), caramel, riboflavin, natural and artificial flavoring and coloring agents (these are the labeled ingredients of the product company on the cans). Omega-3 is available in the form of liquid syrub 120 ml. composition per 5 ml: high DHA Fish oil 640 mg, Rigel evening primrose oil 213 mg, Dl-alpha tocopherol acetate, thyme oil 0.40 mg, equivalent to vitamin E 7.82 mg. manufactured by Sigma pharmaceutical industries. Animals The present study was carried out in the Animal House of Faculty of Medicine, Menoufia University. It included 50 male albino rats weighting 200-250g. They were housed in four hygienic stainless steel cages and kept in clean well-ventilated room. They were allowed free access to water and fed ad libitium. Strict care and hygiene were taken to maintain normal and healthy environment for all rats all time. The general conditions and behavior of the animals were noticed. The animals were divided into four main groups: Group I (control group): Included 10 animals received 2ml distilled water by oral route for 4weeks. Group II (omega-3 treated group): Included 10 animals. They received omega-3 at a dose of 600 mg /kg/ day orally for 4 weeks. Group III (Energy drink treated group): Included 20 animals. They received Red Bull at a dose of 20 mg/kg/day orally for 4 weeks [30]. Half the animals were sacrificed at the end of the period of treatment (Subgroup III A) and the other half was left for another 4weeks (Subgroup III B) Group IV (Energy drink and Omega-3 group): Included 10 animals. They received Red Bull at a dose of 20mg/kg/day and Omega-3 at a dose of 600mg/kg/day for 4 weeks. Sampling, Sectioning and Staining All rats were weighed at the end. During the last day of the experiment, animals were deprived of food overnight then sacrificed by cervical dislocation. Blood samples were taken directly from the heart for biochemical assessment. The abdomen of the rats was opened, the pancreas was dissected out then perfused with cold saline, in 10% neutral buffered formalin overnight then processed to obtain paraffin blocks.Serial Paraffin sections of 5-6 µm thickness were cut and prepared for histological (Hx & E and Toluidine blue) and histochemical (Mallory trichrome for detection of collagen and Gomori for illustration of B and α cells of pancreas) studies. Immunohistochemistry (IHC) assessment Some sections from all specimens (both control and treated) were picked upon coated slides for the immunohistochemical study which was done by peroxidase-labeled Streptavidin-Biotin technique using the anti-insulin antibody; insulin Ab-6 (INS04 + INS05) Mouse Monoclonal Antibody (Thermo Fisher Scientific, Fremont, CA, USA) which is a cocktail especially designed for sensitive detection of insulin. Morphometric study By using Image analyzer software (Image J 1.47v national institute of health, USA) we calculated the percentage area of connective tissue and the intensity of the brown color of anti-insulin immune expression. Statistical analysis Statistical analysis was performed using SPSS software, version 16.00 (Chicago, Illinois, USA). All data were expressed as mean±SD. One-way analysis of variance (ANOVA) and post-hoc with least significant difference were used for comparison between groups. Significance was considered at $p<0.05$

RESULTS I – statistical results A) The body weight There was no significant change in the mean body weights of rats of all studied groups as compared to control group ($P>0.05$). Also, no significant change in the mean body weights of rats of recovery group as compared to protected group. B) The pancreatic weight There was significant increase in the mean pancreatic weight of treated group (p0.05) . Highly significant decrease in the mean pancreatic weight of the recovery group compared to the protected group was observed and group II: Pancreatic sections of control and omega-3 treated groups were almost similar showing different sized lobules composed of serous acini with apical acidophilia and basal basophilia. Intact intra lobular ducts. Islets of Langerhans appeared as pale staining areas of polygonal cells arranged in clusters . There were abundant zymogen granules in acinar cytoplasm .The lobules were separated by thin delicate connective tissue septae with deposition of minimal amount of collagen fibers around ducts and blood vessels . Modified gomori aldehyde fuchsine stain showed normal intensely stained purple to violet beta cell filling the majority of the islet and alpha cells stained yellow

Group III (Energy drink treated group): Pancreatic sections of rats treated with energy drink for 4 weeks then sacrificed 24 hours after the last dose (subgroup IIIA) showed distorted archeticture with small atrophic acini and decreased apical acidophilia. Some nuclei were small pycnotic or even lost and the others showed perinuclear haloes. Ducts were dilated with retained secretions and lined by degenerated epithelium. There were dilated congested blood vessels. Some islets of Langerhans showed apparent increase in size with darkly stained nuclei and others showed degeneration with loss of cells and empty spaces. Zymogen granules were lost. Pancreatic septae are thickened with increased amount of collagen fibers around ducts and blood

Group IV (Energy drink and Omega-3 group): pancreatic sections of this group revealed a noticeable protection of the acini against the deleterious changes induced by the energy drink. The ducts and the islets of Langerhans also appeared nearly normal. There were still dilated congested blood vessels. Zymogen granules were present in acinar cytoplasm. Pancreatic septae was thin with deposition of minimal amount of collagen fibers around ducts and blood vessels. Modified gomori aldehyde fuchsine stain showed normal intensely stained purple to violet beta cell and yellow stained alpha cells

Discussion

Energy drinks refers to dietetic food products in which the main source of energy are carbohydrates, whose energy value is not lesser than 80 kJ/100 ml (19 kcal/100 ml).The main active constituents of energy drinks include varying amounts of caffeine, guarana extract, taurine and ginseng. Also, additional amino acids, vitamins and carbohydrates are usually present. The intended effects of energy drinks are to provide sustenance and improve performance, concentration and endurance [16]. The amounts of guarana, taurine, and ginseng found in popular energy drinks are far below the amounts expected to cause either therapeutic benefits or adverse events. However, caffeine and sugar are present in amounts known to cause a variety of adverse health effects [17]. There are increasing reports of caffeine intoxication from energy drinks, and it seems likely that problems with caffeine dependence and withdrawal will also increase. In children and adolescents who are not habitual caffeine users, vulnerability to caffeine intoxication may be markedly increased due to an absence of pharmacological tolerance [18]. Among the fatty acids, it is the omega-3 polyunsaturated fatty acids which possess the most potent immunomodulatory activities, and among the omega-3, those from fish oil eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)—are more biologically potent than _-linolenic acid (ALA). Some of the effects of omega-3 are brought about by modulation of the amount and types of eicosanoids made, and other effects are elicited by eicosanoid-independent mechanisms, including actions upon intracellular signaling pathways, transcription factor activity and gene expression [19]. The present study revealed no significant differences in the body weight of animals administered energy drinks as compared to the control group. This could also be attributed to caffeine which intensifies metabolism and increases energy consumption. Our study demonstrated that energy drinks administration led to a significant increase in blood glucose level simultaneously with strong positive immune reaction to anti insulin monoclonal antibody. This may indicate insulin resistance. A can of Red Bull assures 100% of niacin RDA (Recommended Daily Allowance). This quantity is 331 El Desouky et al. not usually seen as a risk, but it should be considered that high carbohydrate content of Red Bull may amplify niacin's side effects which include insulin resistance [21,22]. Zhou et al[23] and Li et al[24] recorded that niacin supplementation in high carbohydrate diets may be one of the causes of diabetes. In addition, consumption of high levels of sugar causes various detrimental effects on health, especially inducing insulin resistance, which is closely associated with the development of metabolic disorders such as obesity or type 2 diabetes [25,26]. Also, high levels of blood glucose may cause oxidative stress through the overproduction of reactive oxygen species (ROS)[26]. Under normal conditions, a natural system of scavengers called endogenous antioxidants counteracts the cytotoxicity of ROS produced from molecular oxygen in the mitochondria. Oxidative stress is the molecular and cellular damage resulting from excessive ROS production or from reduced endogenous antioxidants [27,28]. Also, Robertson [29] reported that oxidative stress plays a key role in causing insulin resistance and βeta cells dysfunction by their ability to activate stress sensitive signaling pathways. The pancreas

may be more susceptible to oxidative stress than other tissues and organs because pancreatic islets cells show extremely weak manifestation of antioxidative enzymes [30]. Furthermore, some studies have proved that caffeine may play an important role in the regulation of insulin release and related metabolic disorders. González Dominguez et al[25] showed that healthy young adults who consumed sugar-sweetened drinks with caffeine had a significant increase in blood glucose and insulin levels. This could be synergic effect of caffeine and sugar. Also the metabolic effects observed after caffeine administration could result from its influence on the secretion of stress hormones, including adrenaline and cortisol which provide energy sources necessary to survive the stress. Both adrenaline and cortisol enhance lipolysis and increase plasma concentration of glucose. They additionally enhance gluconeogenesis and reduce peripheral glucose consumption by inhibiting the activity of glycolytic enzymes as a result of increasing the concentration of fatty acids in blood [16]. In this study pancreatic sections of energy drink treated rats showed distorted architecture and variable degrees of acinar and islets' cells degeneration in the form of small pyknotic nuclei or even lost nuclei, intra cellular vacuolations, decreased zymogen granules, fatty deposition and dilated congested blood vessels. These changes are due to the interaction between the different contents of energy drink specially high content of caffeine which induces a pro-oxidant environment [15]. Similar changes have been observed by khayyat et al.[31] on liver tissue after 4 weeks of energy drinks administration . The nuclear changes may be due to the preservatives added to the energy drinks as sodium benzoate which in combination with ascorbic acid, another common ingredient in energy drinks, they could form the chemical benzene, which is carcinogenic [32]. Congestion of blood vessels and infiltration of leucocytes might be due to different reaction of taurine associated with other active ingredients of the energy drinks as caffeine [31]. The thickened pancreatic septae with deposition of collagen fibers is due to the toxic effect of caffeine as reported by Takesue[33] on his study on the effect of caffeine on wound healing of rat gingiva and revealed increased depositions of fibrin on the underlying connective tissue. These changes disappeared in recovery group due to cessation of the irritant, toxic effect of the energy drinks[34]. The present study revealed that most of these finding were reversible after cessation of the energy drink. This is supported by Akande and Banjoko[34] who reported that the damage done by excessive consumption of caffeinated energy drink is reversible as observed in the results of the blood chemistry analysis and the histopathological study of the organs of animals in the recovery group. However, coadministration of Omega-3 with energy drink showed obvious protection of the pancreatic tissue against the hazardous effect of energy drink on pancreatic sections of rats. This may be attributed to its antiinflammatory and antioxidant activity of Omega-3[15]. Erayk et al.[35] reported that Omega-3 fatty acids showed potential effects in lowering blood glucose levels and improving lipid profile and insulin resistance when it was used in combination with Pioglitazone through modulation of Toll-like receptor 4(TLR-4) in type 2 diabetes mellitus. The combined regimen significantly increased the percentage of β-cell area in the pancreatic islets, significantly decreased endoplasmic reticulum stress, and reduced the percentage of apoptotic cell death in the pancreatic islets. These findings suggest that fish oil and/or pioglitazone prevents β-cell dysfunction by improving the insulin resistance and decreasing the ER stress. The anti-inflammatory aspects of Omega-3 fatty acids is relative to prostaglandins and cytokines and their clinical effects in inflammatory and autoimmune diseases. It decreases production of prostaglandin E2 (PGE2) metabolites and increases prostacyclin PGI3, leading to an overall increase in total prostacyclin by increasing PGI3 without a decrease in PGI2 (both PGI2 and PGI3 are active vasodilators and inhibitors of platelet aggregation).Omega-3 also decreases thromboxane A2, potent platelet aggregator and vasoconstrictor whilei ncreasing thromboxane A3, a weak platelet aggregator and a weak vasoconstrictor. Also, it decreases leukotriene B4 formation, an inducer of inflammation and a powerful inducer of leukocyte chemotaxis and adherence while increasing leukotriene B5, a weak inducer of inflammation and a weak chemotactic agent[19]. The present study showed the toxic effects of energy drinks on pancreas and the protective effects of omega-3. More studies are recommended in the future to show the hazardous effects of energy drinks consumption on longer period of time and on different body organs

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