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Effect of Consumption of Caffeinated Energy Drinks on the Myocardium and the Aorta of the Adult Male Albino Rat (A Histological and Morphometric study)

Thesis

Submitted for partial fulfillment of Master Degree in Anatomy and Embryology

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Effect of Consumption of Caffeinated Energy Drinks on the Myocardium of the Adult Male Albino Rat
(A Histological and Morphometric study)

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ABSTRACT

Background: Energy drinks are non-alcoholic beverages containing mainly caffeine. Chronic consumption of energy drinks may be associated with increased blood pressure, and cardiac work load, as well as, increased heart rate. Energy drinks consumption has been related to myocardial infarction in healthy young adult.

Aim of the Work: The aim of the present work was to study the harmful effect of energy drinks histologically and morphometrically, on the myocardium of the adult male albino rats.

Material and methods:

20 Adult male albino rats aging 4-6 moths were divided randomly into two groups; Group I (Control group) which consisted of 10 male albino rats with free access to food (rat chew) and water and Group II (Experimental group): 10 male albino rats which received 3.75 ml/kg body weight red bull through oral gavage daily for 4 weeks with free access to food (rat chew) and water**.** At the end of experiment, rats were scarificed, and cardiac muscle was extracted and processed into paraffin blocks for light microscopic examination. Morphometric study and statistical analysis were done.

Results: The present work demonstrated that Red Bull induced several histopathological changes of the cardiac muscle. The results showed the cardiac muscle of rats with irregularities in the arrangement of fibers and wide spaces in between. Some cardiomyocytes showed areas of pale stained sarcoplasm, partial loss of the cross striations and ill-defined intercalated discs. Localized areas of hyaline degeneration and areas of tissue disruption were also noticed with less frequently localized coalesced cytoplasmic vacuoles. The nuclei of the cardiomyocytes appeared small, darkly stained, and pyknotic. Others were flattened and deeply stained. Congested, and thick walled blood vessels were also observed, with mononuclear cellular infiltrations. In Masson's Trichrome sections, relative increase in collagen deposition between the cardiac muscle fibers was observed which was associated with focal fibrous degeneration. Examination of the semithin sections showed partial disruption of the cardiomyocytes. The nuclei of the cardiomyocytes were small, darkly stained with perinuclear degeneration .Some nuclei were eccentric, or with an irregular outline, and peripheral chromatin condensation. The characteristic finding was the presence of longitudinal wavy cardiac muscle fibers with relatively wide spaces in between and localized areas of partially lost cross striations and intercalated discs. Dilated, thick walled blood vessels was noticed between the bundles of cardiac muscle. Mononuclear inflammatory cellular infiltrations were also detected.

Conclusion: The present study demonstrated the degenerative and cardiotoxic effects of Red Bull as an example of energy drinks on the histological structure of the cardiac muscle.

Keywords: Rat, cardiac muscle, Energy drinks, Red bull, Histomorphometry.

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List of abbreviations

Abb.		Full Term
AV	**:**	Atrioventricular valves
B.C.	**:**	Before Christ
B.W.	:	Body weight
CA	:	Cornu ammonis
Cm	:	Centimetres
CVD	**:**	Cardiovascular disease
DPB	**:**	Diastolic blood pressure
ED	**:**	Energy drink
EDs	**:**	Energy drinks
G	**:**	Grams
GIT	**:**	Gastrointestinal tract
Hx & E	**:**	Hematoxylin and eosin
Kg	:	Kilogram
L	:	Litre
Mg	:	Milligram
Ml	:	Millilitre
mm^3	**:**	Cubic millimetres
Oz	**:**	Ounces
RB	**:**	Red Bull
RDA	:	Recommended daily allowance
SBP	**:**	Systolic blood pressure
ST	**:**	Seminefrous tubules
µm	**:**	Micrometers

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Introduction

Globally, the popularity of energy drinks (EDs) is steadily increasing, especially among athletes, teenagers, and college students **(Alsunni, 2015)**. Energy drinks are non-alcoholic beverages containing mainly caffeine. "Gives you wings" is the promises that energy drinks companies attract their consumers with **(Militante & Lombardini, 2002)**. However, a controversy has been developed regarding the benefits and possible adverse effects of energy drinks **(Haroun, 2019)**.

Energy drinks have been known as healthy drinks within many populations **(Salih et al., 2018)**. Few beneficial effects of energy drinks are reported as they are frequently consumed by the youth to overcome fatigue and sleepiness for studying during the exams periods, increase energy and enhance mental alertness and physical performance **(Ishak et al., 2012)**.

Moreover, moderate doses of caffeine-containing energy drinks have been shown to improve attention, memory, speed reaction times, facilitate vigilance, and improve verbal reasoning **(Curran & Marczinski, 2017)**.

On the other hand, experiments on albino rats using energy drinks, have shown significant elevations of the serum levels of insulin and glucose **(Ayoub & El Beshbeishy, 2016)**.

In male Wister rats, long-term consumption of energy drinks had significantly increased the cardiac glucose and

glycogen levels, the total protein level as well as the ALT and AST enzymes **(Munteanu et al., 2018)**.

Additionally, there were several histological changes like fatty degeneration of hepatocytes, degeneration of renal glomeruli with dilated Bowman's capsules in the renal cortex, pyknosis and chromatolysis of cerebral and medullary neurons, and alterations in the cerebellar Purkinje cells **(Al-Basher et al., 2018)**.

Meanwhile, scientific interest in the effects of energy drinks on humans is also expanding. Chronic consumption of energy drinks may be associated with headache, poor mental health, tachycardia, breathlessness, polyuria, metabolic and renal disorders, and obesity **(Sankararaman et al., 2018)**.

Most of the studies carried out in the general population using EDs, report increased blood pressure, cardiac work load, as well as, increased heart rate **(Bailey et al., 2014)**. Energy drinks alter electrolytes and result in repolarization abnormalities leading to cardiac arrhythmias, myocardial infarction, and prolonged QT interval **(Mangi et al., 2017)**.

Moreover, energy drinks consumption has been related to myocardial infarction in healthy 17-and 19-year old boys **(Wilson et al., 2012)**. This observation has been supported by the findings that consuming energy drinks reduces endothelial function and stimulates platelet activity through arachidonic acid-induced platelet aggregation in healthy young adults **(Pommerening et al., 2015)**. Other reports have demonstrated a relationship between energy drinks overconsumption and arterial dilatation, aneurysm formation,

dissection and rupture of large arteries **(González et al., 2014)**.

Reviewing the literature, it was noted that most of the researchers studied or investigated the physiological and biochemical changes in the cardiac muscle and the aortic tissue that occur with the use of energy drinks, while few described histological or structural changes in experimental animals. Accordingly, it was important to make further studies, especially from the histological point of view to broaden the spectrum of their harmful effects on the cardiovascular system.

Aim of the Work

The aim of the current work was to study the harmful effect of energy drinks consumption, histologically and morphometrically, on the left ventricular myocardium and the ascending aorta of the adult male albino rat, to highlight the extent of impact of energy drinks on the cardiovascular system.

Objectives of the current study was to:

1- Describe the histological changes of rats' left ventricular myocardium and ascending aorta after exposure to energy drinks.

2- Evaluate the morphometric parameters, regarding the mean cross sectional diameter of the left ventricular cardiomyocytes, the mean percentage area of collagen fibers deposition in the left ventricle, the mean total thickness of the ascending aorta, the mean thickness of the tunica media of the ascending aorta and the mean percentage area of collagen fibers deposition in the ascending aorta.

Review of Literature

Anatomy of the Human Heart:

The history of the cardiac anatomy dates back to 3500 B.C., when the Greeks and the Egyptians based their understanding of this structure on their religious beliefs **(Standring, 2016)**.

The human heart is located within the thoracic cavity, posterior to the sternum and costal cartilages, and rests on the superior surface of the diaphragm, lying in the middle mediastinum between the lungs at the level of T5- T8 **(Kalaria et al., 2002)**.

The sternocostal surface of the heart is formed by the right ventricle, while the diaphragmatic surface is formed mainly by the right ventricle and a portion of the left ventricle. The superior border is formed by both atria and their auricles, while the inferior border is formed by both ventricles. The left border is formed by the left auricle and left ventricle. The right border is formed by the right atrium **(Moore & Dalley, 2014).**

The surface anatomy of the heart is represented by an upper border which follows a line from the inferior border of the left second costal cartilage to the superior border of the right costal cartilage. The inferior border of the heart lies along a line from the right sixth costal cartilage to the fifth intercostal space, at the midclavicular line where the apex of

the heart is located. The right and left borders follow lines connecting the right and left ends of the superior and inferior borders (**Weinhaus & Roberts, 2005).**

A double-layer, fluid-filled fibrous sac known as the pericardium, encloses the heart and great vessels. It keeps the heart in a stable location in the mediastinum, facilitates its movements, and separates it from the lungs and other mediastinal structures. The pericardium consists of two layers: the outer fibrous layer and the inner serous layer. The fibrous pericardium is a conical-shaped sac. Its apex is fused with the roots of the great vessels at the base of the heart. Its broad base overlies the central fibrous area of the diaphragm with which it is fused (**Mahabadi et al., 2021).**

The serous pericardium is a layer of serosa that lines the fibrous pericardium, which is reflected around the roots of the great vessels to cover the entire surface of the heart. Between the parietal and visceral layers is a potential space that is filled with a small amount of fluid. The part of the visceral layer that covers the heart, but not the great vessels is called the epicardium (**Volpe & Makaryus, 2020).**

The heart is a remarkably complex organ. It has four chambers arranged in two functional pump series: a right pump comprising the right atrium and ventricle that receives venous blood from the systemic circulation and pumps it on to the lungs for oxygenation, and a left pump comprising the left atrium and ventricle that receives oxygenated blood from the pulmonary circulation and pumps it into the systemic circulation **(Murphy & Lloyd, 2013)**.

The atria function primarily as receiving chambers for blood returning to the heart but also contain the primary cardiac pacemaker, the sinoatrial node, in the wall of the right atrium. The atria actively pump blood to fill the ventricles during the latter part of the ventricular diastole. The anatomical base of the heart is formed mainly by the left atrium receiving the pulmonary veins and to a small extent by the posterior part of the right atrium. Internally, the atrial surfaces are predominantly smooth but have a trabeculated portion in their atrial appendages which represents the primitive embryonic atrium. The smooth portions are incorporated from primitive veins as the embryonic heart enlarges **(Sinnatamby, 2011)**.

The ventricles are the primary pumping chambers and have thick muscular walls. They receive blood from the atria, and pump it to the pulmonary circulation via the pulmonary artery and systemic circulations via the aorta. The internal surface of the ventricular walls have prominent trabeculae carnae and are only smooth in their outflow tracts **(Murphy & Lloyd, 2013)**.

Valves are an important component of the heart. Not only do they act as an exit gate, but they also prevent backflow into the chamber. The aortic valve, separating the aorta from the left ventricle, and the pulmonary valve, separating the pulmonary artery from the right ventricle, are known as semilunar valves. The two atrioventricular (AV) valves are the tricuspid and mitral valves. The tricuspid valve marks the separation between the right atrium and ventricle