

THE EFFECT OF ENERGY DRINK ON THE PANCREAS OF WISTAR ALBINO RATS - A MICROSCOPIC STUDY

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ABSTRACT

Objective: To observe the microscopic changes on the pancreas of Wistar Albino rats, following the oral administration of energy drink.

Study Design: Laboratory based experimental study.

Place and Duration of Study: This study was conducted at the department of Anatomy, Basic medical Sciences Institute (BMSI), Jinnah Postgraduate medical Center (JPMC) Karachi, in October 2018 for a period of four weeks.

Methodology: Thirty adult, healthy male Wistar Albino rats equally divided into three groups were used in the study. Group A served as control, group B as treated with energy drink at a dose of 7.5ml/day orally and group C treated with energy drink at a dose of 15ml/day orally, for a period of 4 weeks.

Results: The Haematoxylin and Eosin (H&E) stained sections of pancreatic tissue have shown marked pathological changes. Dilated and congested blood vessels were a significant finding in the treated tissues. Sequestration of mononuclear cells was also found on microscopy depicting the inflammatory process in the pancreas of treated animals.

Conclusion: Energy drink consumption caused significant injurious and deleterious effects on the pancreas of the Albino rats.

Keywords: Energy drink, Hemorrhage, Mononuclear cells sequestration, Vascular congestion.

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INTRODUCTION

The consumption of energy drinks is a matter of debate nowadays. Having an accurate knowledge regarding its ingredients and safety levels is a must for its consumers.

Energy drinks are the popular alcohol free beverages having a combination of high dose of caffeine along with sugar, vitamins, and various herbs like guarana and ginseng biloba, all present in varied amounts^{1,2}. The major users of these drinks are children and young adults between 10-18 year of age³ and are being maximally affected by the hazardous effects of these beverages⁴. People use it generally to increase the attention span, maintain the state of arousal during prolonged hours of fatigue, improve the cognitive performance or while partying with friends⁵.

Chronic usage of caffeine has many

unwanted effects on many organ systems of the body. It badly affects the central nervous system, cardiovascular system, gastrointestinal tract and renal functions⁶ causing delirium, seizures, tremors⁷, arrhythmias⁸, increase gastrointestinal motility and frequency of micturition. Experimental studies have proved that the use of energy drinks is a potent source of derangement of the liver enzymes. A significant increase has been seen in the hepatic enzymes, which is sufficient enough to produce the sign and symptoms of acute hepatitis⁹. It also has been observed that the intake of caffeine in late hours of the day or at night in particularly, has found to affect the natural circadian rhythm of the body. It prolongs the circadian clock and therefore alters the coordination of the body with the alternate light and dark changes in the environment¹⁰.

Pancreas is an organ that also has found to affect greatly by the intake of these non-alcoholic caffeinated beverages. Literature has revealed that the usage of energy boosting beverages

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Received: 25 Jul 2019; revised received: 15 Oct 2019; accepted: 28 Oct 2019

induces the morphological and biochemical alterations, which are reflected by the deranged blood glucose, increase serum amylase and lipase levels. Pancreatitis is also found to be an outcome of intoxication along with decreased insulin sensitivity rendering the tissues less susceptible to absorb glucose, further raising the blood glucose levels and increase chances of diabetes mellitus.

Although very limited literature is available on the effects of these energy enhancing drinks on the pancreas at microscopic level, the current study was therefore aimed to investigate the histological changes induced by energy drinks on the pancreas of Wistar Albino rats.

METHODOLOGY

This laboratory based experimental study was conducted in the department of Anatomy, BMSI, JPMC, Karachi in collaboration with the animal house of JPMC, from September to October 2018, after getting the approval from the ethical committee of BMSI (Lt No. F.1-2/2018/BMSI-E.COMT/069/JPMC dt 28-9-2018).

Thirty adult male Wistar Albino rats (Charles Brooklyn strain) weighing between 250-

week before the commencement of the study to their behavior, health status and amount of diet intake. These animals were housed in the experimental room of the animal house and maintained on the standard laboratory diet and water ad libitum and were exposed for 12 hour day and night cycle at 30°C. The rats were equally divided into three groups A, B and C (n=10), after a week-long adaptation.

The groupings of animals were as follows:

Group A = served as control, on standard laboratory diet

Group B= Energy drink treated at a dose of 10mg/kg equivalent to 7.5ml/day

Group C= Energy drink treated at a dose of 20mg/kg equivalent to 15ml/day

Commercially packaged cans containing 250 ml of the commonly available energy drink in Pakistan, was used for the study (identity of the drink has been hidden for legal protection). The route of administration was oral via the gastric tube at a dose of 7.5ml and 15ml given daily to all the animals of group B and C respectively for a period of 30 days.

Table: Comparison of pathological changes within groups A, B, C (n=10).

	Group A (Control)	Group B (Treated, Low Dose)	Group C (Treated, High Dose)	p-value
Vascular Congestion				
None	9 (90%)	1 (10%)	1 (10%)	0.001**
Mild	1 (10%)	2 (20%)	-	
Moderate	-	5 (50%)	2 (20%)	
Severe	-	2 (20%)	7 (70%)	
Mononuclear Cell Infiltration				
None	10(100%)	2 (20%)	-	0.05*
Mild	-	3 (30%)	1 (10%)	
Moderate	-	4 (40%)	2 (20%)	
Severe	-	1 (10%)	7 (70%)	
Edema				
None	10 (100%)	3 (30%)	1 (10%)	0.05*
Mild	-	7 (70%)	9 (90%)	

300 grams were selected for this prospective study. Selection criteria based on non-probability (purposive) simple random sampling. The sample size was calculated by open Epi web based calculator. The animals were observed a

All the animals were sacrificed at the completion of experimental period. The pancreas were removed and were fixed in 10% buffered neutral formalin (BNF) for 24 hours. The tissues were then dehydrated through ascending grades

of alcohol, cleared in xylene, infiltrated in paraffin at 59 and paraffin embedded blocks were made.

Tissues were sectioned at 5µm thickness and stained with Haematoxylin and Eosin (H&E) microscopy. Four randomly selected, non overlapping sections from each slide were observed under light microscopy at 100X magnification. A comparison was done between the control and the treated animals. The following histopathological findings were observed: vascular congestion, mononuclear cell infiltration and tissue edema. Here, vascular congestion refers to dilatation and engorgement of blood vessels. Mononuclear cell infiltration refers to invasion of tissues primarily by lymphocytes. Tissue edema refers to accumulation of free fluid in tissue spaces¹². The microscopic observations were graded as none, mild, moderate and severe on the basis of presence of the findings in one field was graded as mild, in two or three were labeled as moderate and in all four fields were labeled as severe. The mere absence of these pathological changes was labeled as none¹¹⁻¹³.

The statistical significance of differences in the categorical variables between the experimental and control groups were evaluated by the chi-square test on SPSS-20. The frequency was expressed in percentage. The *p*-value of ≤ 0.05 was considered significant and ≤ 0.001 was considered highly significant.

RESULTS

The pancreatic tissues were carefully observed under the microscope. Both the acini as well as the islets of Langerhans were subjected to thorough histological examination.

Microscopic examination of group A animals revealed the regular pattern and the normal cytoarchitecture of the parenchyma of pancreas. The pancreatic acini had intact margins with pale cytoplasm and darkly stained nuclei. The islets were appeared as more or less spheroidal masses of cells arranged in the form of anastomosing cords (table, fig-1 & 4).

The pancreatic tissue of treated group B showed mild to moderate injurious effects. Few lymphocytes were observed in the parenchyma. Moderate amount of vascular congestion along

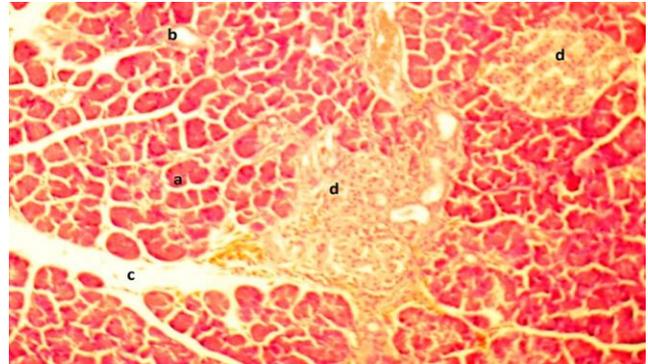


Figure-1: Control group A pancreas showing a. Acini b. Blood vessel c. Connective tissue septa d. Islet of Langerhans (H & E 100X).

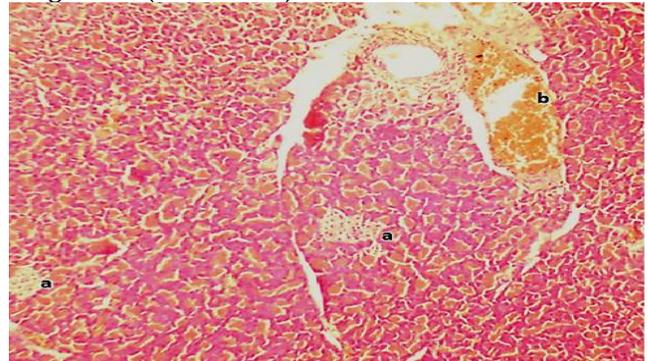


Figure-2: Treated group B showing a. Shrunken islets and b. Hemorrhagic vessel (H & E 100X).

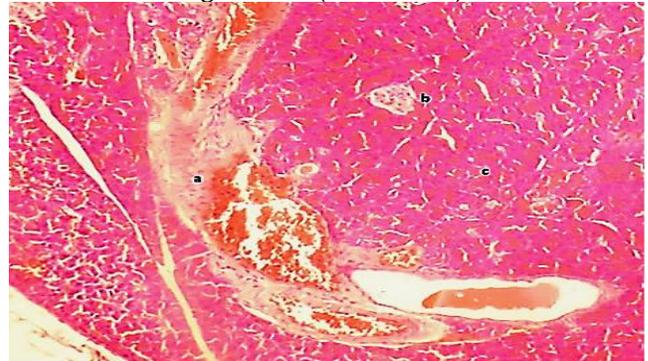


Figure-3: Treated group C showing a. Dilated and congested blood vessels, b. Shrunken islet and c. Distorted architecture of pancreatic acini (H & E100X).

with hemorrhage and mild edema was found in this group (table, fig-2 & 4).

The histopathological changes were more marked in group C as compared to group B

tissues. Microscopic examination exhibit disruption of parenchyma with widened intralobular spaces. Severe vascular congestion with dilatation and hemorrhage was seen. Mononuclear cells predominantly lymphocytes were observed in the parenchyma as well as surrounding the blood vessels. Gross shrinkage of islets and acini were also observed (table, fig-3 & 4).

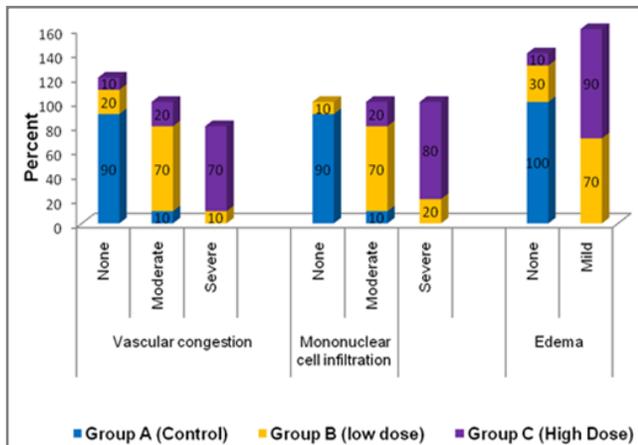


Figure-4: Comparison of pathological changes within groups A, B, C.

DISCUSSION

Energy drinks contains a high amount of caffeine which induces a pro oxidant environment. It damages the tissues by generating the reactive oxygen species (ROS)¹¹. Studies have revealed that generation of ROS to a limited extent is a usual response of inflammation. But if their production encompasses the normal capacity, it results in the cellular dysfunction and tissue injury^{14,15}. Pancreas is a very sensitive organ with very limited regenerating capacity. Prolonged exposure to the inflammatory mediators has damaging effects on the vascular and ductal part of the organ with loss of islet cell function¹⁶.

The current study was intended to explore the histological changes induced by the intake of energy boosting beverages at different doses on the pancreas of Wistar Albino rats. These changes were more remarkable in high dose treated animals. Vascular congestion with hemorrhage, mononuclear cell infiltration and edema were observed as a part of microscopic examination.

The pancreatic tissue of high dose treated animals showed moderate to severe influx of mononuclear cells largely lymphocytes, scattered among the parenchyma of the organ. Ayoub and El Beshbeishy (2016)¹¹ have also reported the inflow of mononuclear cells which supports the observations of the current study.

Bukhari *et al*¹⁷ and Salih *et al*¹⁸ have found significant edema in hepatic and renal tissues treated with energy enhancers. Existing study was however in contradiction to the above given findings where mild tissue edema has been observed in the treated animals.

A dose dependent increase in the vascular congestion and dilatation were found in present study. Low dose treated animals showed moderate degree of engorged and thickened blood vessels while animals treated on high dose exhibit severely dilated, congested and hemorrhagic vessels. Similar results have been stated by Khayyat *et al*¹⁹ and Ayuob and El Beshbeishy¹¹ in hepatic and pancreatic tissues respectively following the administration of caffeinated beverages. Pober and Sessa²⁰ have described that the damage to the endothelium of blood vessels leads to the sequestration of lymphocytes in response to an injury, resulting in the release of chemical mediators. This event plays an integral role in the inflammatory response against a potentially toxic substance in the body.

On the basis of the findings of the current study, it may be stated that consumption of energy boosting beverages has many injurious and deleterious effects on the normal structure of pancreas and thus, its usage must be strictly regulated.

Funding Source

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONCLUSION

The use of energy drinks has found to produce significant changes in the normal cytoarchitecture of pancreas. The results of the

current study can be taken into account in order to make the preventive strategies in future.

Author's Contribution

Dr. Fatima Rehman: Focal person to conceive the idea to conduct the research and data collection, Dr. Zia-ul-Islam: Supervision of the project and Literature search, Dr. Uzma Hameed: Data feeding and analysis and Dr. Sadia Rehman: Drafting of the article and statistical expertise.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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