



ORIGINAL ARTICLE

## Histomorphometric effects of energy drink consumption on pancreatic tissue of albino wistar rats.

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**ABSTRACT... Objective:** To determine the histo-morphometric effects of energy drink consumption in different doses on the pancreatic tissue of Wistar Albino rats. **Study Design:** Experimental study. **Setting:** Department of Pharmacology, Isra University, Hyderabad. **Period:** April 2021 to September 2021. **Material & Methods:** Thirty healthy albino Wistar rats of body weight  $200 \pm 20$  were included in the study. Rats were divided equally into three groups. Group I (Control), Group II (Low dose energy drink 7.5 ml/day) and Group III (High dose energy drink 15 ml/day). The body weight of all animals was measured. Blood samples were collected for the biochemical analysis including serum glucose, serum insulin level, and oxidative markers. Histo-pathological and morphometric findings were observed. **Results:** A statistically significant rise in body weight in groups II and III ( $p < 0.05$ ). Significantly increased serum glucose and declined insulin level was seen in group II and III compared with controls ( $p < 0.05$ ). There was a significantly lower level of oxidative markers observed in group III than in groups I and II ( $p < 0.05$ ). The mean pancreatic acini and islets diameter in groups II and III has decreased significantly compared with group I ( $p < 0.05$ ). The histo-pathological grading reveals that mild to moderate parenchymal changes in the pancreatic tissue of group II rats compared with group I. While moderate to severe changes were observed in group III compared with group I and II rats. **Conclusion:** Caffeinated energy drink consumption results in oxidative stress and degenerative changes in the potential to seriously alter the exocrine and endocrine pancreas' normal morphology consumption.

**Key words:** Energy Drinks, Oxidative Stress, Pancreas.

### INTRODUCTION

Energy drinks (EDs) are popular and universally consumed alcohol-free drinks globally famous among children, adolescents and adults, between the ages of 10 and 35 years. EDs are designed to provide the user with a cocktail of stimulating substances and energy boosters that upsurge physical endurance and focus, enhance mood, and improve cognitive and muscular performance.<sup>1</sup> These drinks are high in caffeine content in addition to water, nicotinamide, carbohydrates, taurine, vitamin B-complex, riboflavin, pyridoxine, artificial sweeteners and a variety of herbs including the derivatives of guarana and Ginseng Biloba are also present as active ingredients.<sup>2</sup>

Caffeinated E.Ds intake over an extended period of time has numerous negative effects on the body's organ systems. Additionally, the higher amount of sugar content in EDs causes obesity and diabetes. On the other hand, taurine (a non-essential amino acid) is another ingredient in these drinks. Both sugar and taurine may have negative effects on the central nervous, cardiovascular, gastrointestinal, renal heart, and even skeletal systems.<sup>2,3</sup> This taurine can result in cardiac arrhythmias, neurological deficits like mood swings, behavioural disorders, delirium, insomnia, nervousness, restlessness, seizures and autism. While increased gastrointestinal motility gastric irritation, nausea, vomiting, and increased frequency of micturition are also associated with taurine imbalances.<sup>4,5</sup> Moreover,

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consuming these caffeinated EDs late at night or during the day, in particular, has been found to interfere with the body's normal circadian rhythm. This results in delaying the circadian clock causing disruption in the body's ability to coordinate with the alternating changes in light and dark in the environment.<sup>6</sup>

Different experimental studies and research in order to assess and comprehend the impact of EDs on various body organs. Studies have revealed that the consumption of EDs is a significant source of hepatic enzyme derangement that increases their levels significantly and produces symptoms of acute hepatitis.<sup>7,8</sup> While some experimental studies have also reported that imbibing caffeinated beverages can disrupt the normal morphology as well as the physiology of the pancreatic tissue. Along with the functional issues, it also damages the organ's exocrine and endocrine components.<sup>9,10</sup>

All the untoward effects on different body organs of the younger generation due to the growing consumption of caffeinated beverages in this age group need to be determined. Various studies have been done to demonstrate and explain the toxic effects of different ingredients of EDs on various body organs. The present study was designed with the objective to determine the histo-morphometric effects of EDs consumption at different doses on the pancreatic tissue of Wistar Albino Rats.

## MATERIAL & METHODS

This experimental study was conducted in the department of pharmacology at Isra University, Hyderabad in April-September 2021. Thirty healthy Wistar albino rats having body weight  $200 \pm 20$  were included in the study. Rats were recruited from the Animal husbandry of Sindh Agricultural University, Tando Jam. For the present study, 250 ml commercially packaged cans of the widely available and used energy drink in Pakistan were used (for the legal protection purpose, the identity is kept hidden).

Animals were handled according to the guidelines for handling laboratory animals provided by the

national institute of health.<sup>11</sup> One week prior to the start all the animals were acclimatized and housed in standard metallic cages with sawdust bedding. Under a 12/12-hour light/dark cycle, the room temperature where the cages were placed was kept in a temperature-controlled environment at  $25 \pm 2^\circ\text{C}$ . All animals were fed on a standard laboratory diet of normal chow and water ad libitum. At the completion of the acclimatization period, the body weight of all animals was recorded by electronic precision balance and then all were divided randomly into three groups, each group comprising 10 animals ( $n=10$ ).<sup>12</sup> Group I (Control), in this group rats, were given a normal laboratory diet (normal chow) and water ad libitum for one month. Animals from experimental groups (groups II and III) received the drinks at doses (of 7.5 ml/day and 15 ml/day respectively) through a gastric tube for a duration of 30 days.

After the completion of duration of experiment, the body weights of all the rats were again measured. All the animals were sacrificed through cervical dislocation. The pancreas of all rats was dissected, and the area-calibrated ocular grid was used to take two measurements for each acinus and islet in each pancreatic section. The maximum transverse and vertical axes were measured, and the mean was computed by adding the maximum transverse and vertical axes and dividing it by two.<sup>13</sup> Later pancreatic tissue was cut up into small pieces and fixed in buffered neutral formalin (10%) for more than 24 hours. After which it was submerged in xylene and then embedded in paraffin wax. The pancreatic tissues were then cut into 4- $\mu\text{m}$  thick slices using a rotary microtome 290. Hematoxylin and eosin (H&E) staining was applied and were examined under a light microscope at 100X and 400X magnifications, the diameters of the pancreatic acini and islets of Langerhans under the light microscope. For determining the histo-pathological alterations and degree of tissue damage, a grading system was adopted. Based on the severity and degree of the alterations, the scale was divided into four categories: none, mild, moderate, and severe.

Blood was drawn by cardiac puncture for

hematological analysis including blood glucose, insulin level, and oxidative markers. The collected samples were then shifted to the laboratory for performing serum analyses that were completed within 24 hours. Plasma was separated from the blood and stored at  $-4^{\circ}\text{C}$  before analysis in a laboratory centrifuge after the blood was spun at 2000g for five minutes. Sera was employed to measure the levels of serum insulin (by the enzyme-linked immunosorbent assay (ELISA) method). ELISA kits were also used to measure the serum concentration of tumor necrosis factor-alpha (TNF- $\alpha$ ). A Nitric oxide (NO) assay kit for the quantitative determination of nitrite and nitrate was used to measure the serum level of NO in accordance with the instructions provided by the manufacturer. As previously described, superoxide dismutase (SOD), and reduced glutathione (GSH) were measured in pancreatic tissue homogenate using kits in accordance with the manufacturer's instructions.<sup>13</sup>

SPSS ver. 23 was used to enter and analyze the data. The data presented was expressed as mean and standard deviation. Post-hoc Tukey's test was used to compare various groups with the control group after the ANOVA test. P-value less than 0.05 was set as level of significance.

The study was ethically approved for the experimentation from the ethical committee of Isra University, Hyderabad (Letter No. IU/RR-10-IRC-21/N/25/1845).

## RESULTS

The mean pre-experiment body weight of group I was  $201.4 \pm 4.6$  grams, group II was  $202.2 \pm 3.7$  grams and group III was  $206.6 \pm 4.5$  grams. The post-experimental body weights are demonstrated in Table-I. There was a statistically significant difference was observed between the experimental groups ( $p < 0.05$ ). (Table-I)

Table-II is demonstrating the morphometric analysis and hematological findings of all three groups. The mean pancreatic acini and islets diameter in groups II and III has decreased significantly compared with group I ( $p < 0.05$ ). Hemotological profile of rats in ED groups (group II and III) revealed a significant decline in levels of serum insulin, GSH and SOD levels ( $p < 0.05$ ) compared with the controls. Whereas, a rising level of serum glucose, NO and TNF- $\alpha$  when compared with the controls. However, this decline in all the mentioned factors and rise in all the mentioned markers are more pronounced in group III rats compared with their counterparts in group II. ( $p < 0.05$ ) (Table-II)

| Groups    | Body Weight     | P-Value |
|-----------|-----------------|---------|
|           | Mean $\pm$ SD   |         |
| Group I   | $204.3 \pm 3.7$ | 0.000*  |
| Group II  | $217.4 \pm 2.7$ |         |
| Group III | $234.6 \pm 3.5$ |         |

**Table-I. Mean  $\pm$  SD body weight (post-experimental)**  
\*: statistically significant ( $p < 0.05$ )

| Morphometric Changes                | Group I               | Group II              | Group III             | P-Value |
|-------------------------------------|-----------------------|-----------------------|-----------------------|---------|
|                                     | Mean $\pm$ SD         | Mean $\pm$ SD         | Mean $\pm$ SD         |         |
| Pancreatic acini diameter           | $26.3 \pm 4.7$        | $12.9 \pm 1.5^{a,c}$  | $9.7 \pm 0.7^{a,b}$   | 0.000*  |
| Pancreatic Islets diameter          | $122.7 \pm 21.1$      | $63.2 \pm 8.8^{a,c}$  | $44.5 \pm 7.7^{a,b}$  | 0.000*  |
| <b>Hematological Analysis</b>       |                       |                       |                       |         |
| Fasting Blood glucose (mg/dl)       | $89.7 \pm 12.8^{b,c}$ | $124.5 \pm 9.2^{a,c}$ | $146.7 \pm 8.8^{a,b}$ | 0.000*  |
| Serum insulin( $\mu\text{l/ml}$ )   | $13.6 \pm 1.6^{b,c}$  | $7.6 \pm 1.1^{a,c}$   | $5.7 \pm 0.9^{a,b}$   | 0.000*  |
| Tumor necrosis factor-alpha (pg/ml) | $52.7 \pm 3.0^b$      | $84.8 \pm 3.5^{a,c}$  | $114.7 \pm 3.8^b$     | 0.000*  |
| Nitric Oxide ( $\mu\text{mol/l}$ )  | $10.9 \pm 1.8^{b,c}$  | $29.8 \pm 3.2^{a,c}$  | $44.3 \pm 4.4^{a,b}$  | 0.000*  |
| Superoxide dismutase (U/mg tissue)  | $56.4 \pm 2.1^b$      | $41.8 \pm 1.9^{a,c}$  | $32.7 \pm 1.8^b$      | 0.000*  |
| Reduced glutathione (U/g tissue)    | $3.8 \pm 0.8^{b,c}$   | $2.5 \pm 1.0^{a,c}$   | $1.4 \pm 0.7^{a,b}$   | 0.000*  |

**Table-II. Mean and SD of Morphometric and hematological analysis of study groups**  
\*:  $p < 0.05$  statistically significant

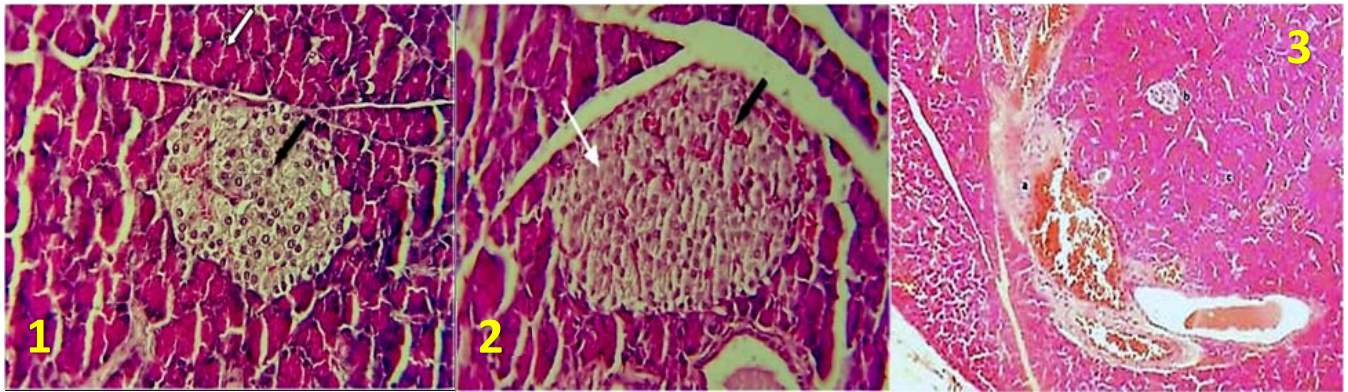


Figure-I. Histo-pathological findings of all groups (groups I, II and III) (n=30)

Figure-1 showing pancreatic acini and normal islets of Langerhans (black arrow and white arrow respectively). Acini and islets were randomly distributed throughout the pancreatic parenchyma, indicating healthy exocrine and endocrine tissues. (H&E X 400). Image (2) showing low dose energy drink group reveals the mild to moderate injurious effects on the tissue. Moderate amount of vascular congestion, haemorrhage, mild edema (black arrow), and necrosis of Langerhans cells (white arrow). (H&E X 400). High dose energy drink group (3) demonstrating disrupted parenchyma with widened intralobular spaces. Severe dilatation with congested blood vessels and hemorrhage (a), gross shrunken islets (b) along with gross distorted architecture of pancreatic acini (c) (H&E100X)

The histo-pathological grading reveals that mild to moderate parenchymal changes in the pancreatic tissue of group II compared with the control group rats. While moderate to severe changes were observed in group III rats compared with group I and II rats. (Table-III)

|           | Vascular Congestion | Mononuclear Cell Infiltration | Edema |
|-----------|---------------------|-------------------------------|-------|
| Group I   | -                   | -                             | -     |
| Group II  | ++                  | ++                            | +     |
| Group III | +++                 | +++                           | ++    |

Table-III. Histopathological grading comparison of pancreatic tissues in all groups of rats  
No (-), mild (+), moderate (++) , severe (+++)

**DISCUSSION**

EDs consumption results in different hazardous

effects on different organs. Consuming caffeinated and sugary EDs encourages insulin resistance resulting in blood sugar levels may increase risk of cardiovascular diseases, obesity and diabetes.<sup>14</sup> The present study mainly focuses on determining the histo-morphometric effects of ED consumption in different doses on the pancreatic tissue of Wistar Albino rats. In this study, the pre and post-experimental body weights of all three group rats were measured. The highest body weight gain (+28%) was observed in group III compared with group I and II rats ( $p < 0.05$ ). Ariffin et al. and Zafar et. al. also reported finding that long-term consumption of EDs is associated with an increase in body weight.<sup>5,15</sup> The preliminary results reported by Mattioli et. al showed that animals administered EDs quickly gained body weight (+12%:  $p < 0.01$ ) compared with sweetened coffee and the control groups. These findings are in agreement with our study.<sup>3</sup>

$\beta$ -cells of pancreatic islets are primarily responsible for insulin secretion, they play an important role in regulating glucose homeostasis in the body, which is the maintenance of glucose at a steady-state level.<sup>16</sup> Our study found that ED caused a dose-dependent considerable upsurge in fasting serum glucose levels ( $p < 0.05$ ) while decreasing insulin levels significantly ( $p < 0.05$ ). Ayuob et. al reported that they administered ED daily for four weeks via a gastric tube, and found a positive correlation



between energy drink consumption and increased blood glucose levels.<sup>17</sup> Moreover, other studies by Rehman et al. and Haroun et al. stated that drinking caffeinated beverages reduces the body's peripheral tissues' sensitivity to insulin, resulting in metabolic consequences.<sup>13,18</sup>

Our study also found a noteworthy ( $p < 0.05$ ) rise in serum NO and TNF- $\alpha$  levels in the high-dose ED consumption rats group compared with controls and low-dose group. While a significant ( $p < 0.05$ ) decline there was a decline in SOD and GSH levels in the pancreatic tissue. The antioxidant enzymes are unquestionably the first line of defence against oxidative stress-related cell damage. SOD is a crucial antioxidant for protecting cells from the oxidative damage resulting from free radicals. The highly reactive superoxide anion is neutralized by SOD by oxidation to hydrogen peroxide.<sup>19</sup> Mansy et al. reported a significant decline in SOD in their rats sample even in lower doses of 1.1 ml and 2.1 ml 100g body weight/day after 4 months.<sup>8</sup> These findings are consistent with the present study. Moreover, findings of Ayuob et al. and Hulail et al. also reported consistent with our study findings.<sup>17,20</sup>

A notable morphometric finding in the current study was the gradual reduction in the diameters of both Langerhan's cells and pancreatic acini. When compared to the animals in the control group, the animals that had received the high dose had significantly smaller islets and acini. Consistent finding are also reported by Hulail et al and Rehman et al.<sup>13,20</sup>

On microscopic examination, edema, mononuclear cell infiltration, and marked vascular congestion with hemorrhage were discovered. These results are following the conclusion given by Shuaib et al., who examined the negative effects of energy drinks on the rat hippocampus and discovered a significant decrease in the diameter of neurons with pyknotic nuclei

and inflammatory infiltrates.<sup>21</sup> Furthermore, it has been noted by Ayuob et al. that excessive consumption of energy drinks can create an environment that is pro-oxidant and leads to an increase in protein oxidation that harm a number of body organs, including the pancreas.<sup>4,17</sup>

In the current study, rats in group 2 received 7.5 ml/kg of ED for a month, which caused the islets of Langerhans to become congested with blood vessels, grow in size, and experience Langerhans cell necrosis. The changes in the pancreatic sections became more noticeable when the ED dosage was raised to 15.0 ml/kg. In addition to the pancreatic acini and islets of Langerhans cells degenerating, there was necrosis of additional islets and infiltration of mononuclear inflammatory cells. After giving rats various doses of energy drinks for 4 weeks, other researchers also noticed these findings in the pancreatic tissue.<sup>3,20,22</sup>

The current study also identifies similar harmful effects that cause harm to the organ's parenchyma, which is manifested by distorted connective tissue architecture, dilated and clogged blood vessels, and shrinkage of the internal organ structures in the endocrine and exocrine parts. Furthermore, vascular congestion and dilatation increased in a dose-dependent manner. While animals treated with low doses of the drug exhibited moderately engorged and thickened blood vessels, those treated with high doses showed severely dilated, congested, and hemorrhagic vessels. Following the administration of caffeinated beverages, similar findings in hepatic and pancreatic tissues have been reported by Al Eyrani et al. and Al Siddiqui et al. respectively.<sup>9,23</sup> This occurrence is crucial to the body's inflammatory response to a substance that could be toxic.

Due to time and financial constraints, other parameters, like lipid profile and more detailed role of different ingredients in ED could not be investigated. Moreover, only pancreatic tissue and its markers were only investigated in single setting. Therefore a study be done to determine the effects of ED consumption, both on its own and when combined with other antioxidants on pancreatic as well as other organs is advised.

## CONCLUSION

The present study concludes that Caffeinated ED consumption results in oxidative stress and degenerative changes in the potential to seriously alter the exocrine and endocrine pancreas' normal morphology consumption.







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| 1   | Shahnaz Bano Memon  | Conception and design of the work, Drafting and final approval of the article.             |  |
| 2   | Sajjad Ali Almani   | Statistical analysis and interpretation of data.   |  |
| 3   | Saqib Baloch        | Drafting of the article, Collection and assembly of data.                                  |  |
| 4   | Shahab Hanif        | Drafting the article, Critical revision of the article for important intellectual content. |  |
| 5   | Ayesha Asad         | Drafting the work and acquisition of data.   |  |
| 6   | Rida Qureshi        | Acquisition of data.   |  |