

Chronic Inflammatory Changes in Esophageal Mucosa of Albino Rats Following Prolonged Intake of Carbonated Drinks

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Abstract

Objective: To study the effects of prolonged intake of carbonated drinks on the oesophagus of albino rats.

Background: Most carbonated soft drinks contain abundant sugars with no alcohol and produce effervescent. Soft drinks containing carbondioxide that are mostly consumed include Pepsi cola, 7-up and mountain dew.

Method: This experimental study was carried out for a period of six months. Seventy five rats of wistar strain (gender non-specific) were divided into two groups i.e. control (A1, A2 & A3) and exposed groups (B,C & D). The controls and the exposed animals were given, the same balanced diet, prepared at the lab's kitchen. The fluid intake of exposed animals was restricted to carbonated drinks only, whereas, the controls were fed water. At the end of the first month, exposed group B and control group A1 were dissected. Similarly, at the end of third month, exposed group C and control group A2 and at the end of six months exposed group D and control group A3 were sacrificed and histological section of oesophagus were obtained for histo-morphological studies.

Results: On micrometry, width of the oesophagus of the exposed animals for one month; exposed Group B was higher ($14.60 \pm 4.43 \mu\text{m}$) than those of Group A1 ($6.2 \pm 1.87 \mu\text{m}$). Similarly, Group C rats which were exposed to carbonated drinks for three months had significantly increased mean esophageal width $9.40 \pm 2.35 \mu\text{m}$ compared to $5.80 \pm 1.81 \mu\text{m}$ mean esophageal width of A2 group. At the end of six months exposure to carbonated drinks, average oesophageal wall thickness $13.60 \pm 2.89 \mu\text{m}$ was found to be more in the exposed group 'D', as compared to $4.50 \pm 1.43 \mu\text{m}$ measured in Control Group A3 ($p < 0.01$).

Conclusion: In conclusion, prolonged consumption of carbonated drinks resulted in gross and histomorphological changes in the esophageal mucosa in the exposed group when compared with the controls. The results of this research will help provide awareness to the general public of the adverse effects afflicted by these beverages.

Key words: Esophagus, mucosa, carbonated drinks, rats, pH

IRB: Approved by the Department of Postgraduate Medical Education, Training, Research and Ethics of Shaheed Mohtarma Benazir Bhutto Medical College. Ref# F-SMBBCL/(ERC)/2020-21/0546. Dated: 12th July 2020

(ASH & KMDC 27 (4):175;2022)

Introduction

In recent years, beverages and processed foods, particularly carbonated soft drinks, have increasingly been included in the typical diets of children, adolescents, and adults. The term "soft drink" refers to a carbonated, industrial product that does not contain alcohol, has pleasant aromas,

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Date of Submission: 3rd November 2022

Date of Acceptance: 29th November 2022

and is highly rehydrating. They are made from sugar that has no nutritional value and contributes to an excessive intake of empty calories. In addition, consumption of carbonated drinks may substitute for nutritious foods that support normal human growth and development, such as milk, natural juices, and fruits¹. Moreover, carbonated drinks contribute to the development of incompetence in the lower esophageal sphincter, a delay in gastric emptying, and abnormalities in the esophageal mucosa, all of which increase the risk of developing reflux of gastric contents into the esophagus. In fact, due to the gastric acid causing tissue damage, persistent oral regurgitation may result in esophageal epithelial changes or the development of gastroesophageal reflux disease¹.

Gastroesophageal reflux disease (GERD) is a common gastrointestinal disorder associated with symptoms of heart burn and regurgitation. Various large scale population-based studies in the Western states have reported a prevalence of gastroesophageal reflux diseases GERD upto 10-20%. The frequency of GERD in Eastern Asia was 5.2-8.5% with more prevalence stated in South Central Asian countries including Iran; 6.3-18.3%. According to multiple hospital based studies on gastroesophageal reflux disease, a prevalence of 22.2-24.0% has been reported in Pakistan. The general health, daily and social functioning, physical and emotional activities, and general health are all affected by GERD. With frequent interruptions during sleep, work, and social activities, it has a significant impact on health-related quality of life. A condition known as GERD occurs when stomach acid reflux causes troublesome symptoms and/or complications. Symptoms are deemed "troublesome" when they have a negative impact on an individual's health².

Gastroesophageal reflux disease has a prevalence rate of 26.6%, according to a cross-sectional study in Pakistan's Southern Punjab. The study looked at risk factors and frequency of the condition. The most typical signs of GERD are acid regurgitation and heartburn. Asthma, dental erosions, reflux chest pain, trouble sleeping, cough, hoarseness, and other oesophageal and extra-oesophageal symptoms and syndromes are all examples. Additionally, the serious complications of GERD include Barrett's esophagus, esophageal stricture, and esophageal adenocarcinoma. GERD can lead to serious complications, morbidities, and financial burdens if left untreated, necessitating additional lifestyle changes, long-term management plans, and surgical interventions. Gastric distention caused by liquid or gas boluses in the carbonated drinks may also temporarily relax the lower oesophageal sphincter, increasing the likelihood of reflux episodes, according to previous research. In spite of the fact that, reflux of fluid stomach contents is probably going to bring about quantifiable reflux, past speculation has additionally recommended that the retr-

ograde development of gas boluses could likewise disrupt the mucosal lining of the digestive tract. Thus, the digestive system when exposed to carbonated drinks cause repeated assault on the mucosa resulting in histomorphological changes of varying intensity which resulted in far reaching effects on the functioning of the different parts of the system³.

Acid exposure in the proximal portion of the esophagus causes symptoms earlier than acid exposure in the distal portion. However, enhanced sensitivity and impairment of mucosal integrity is found more in the distal portion of the esophagus than the proximal portion⁴.

The carbonated drinks with an extremely low pH 2.5 when regurgitate into the esophagus due to lower esophageal sphincter incompetence admixed with gastric juices will play havoc with the mucosa at the lower end due to its physical and chemical structure thereby resulting in growth of microbial flora, harmful to the tissue⁵.

Thus, the main aim of this study is to observe gross and histomorphological changes in the esophageal mucosa after prolonged consumption of carbonated drinks. The rationale of this research is to provide awareness to the general public of the adverse effects afflicted by these unhealthy beverages on the human body.

Patients and Methods

This experimental study was carried out for a period of six months. Seventy five rats of wistar strain (gender non-specific) were divided into two groups i.e. Control labelled as A group which was further subdivided into A1, A2 & A3 groups and three Exposed groups as B,C and D. After approval from the institutional research committee of Shaheed Mohtarma Benazir Bhutto Medical College, the project was initiated. The animals were procured from the animal house and kept in the laboratory in cages and observed for a week according to the guidelines of animal care⁶. They were observed to rule out ill health, loss of weight or lethargy in general. An animal with such an issue was removed from the group and fresh animal was

introduced. They were fed proper diet made in the galley by trained cook and water was fed to them prior to the start of experiment. Thence, water was replaced with carbonated drinks (for the exposed animals) till the end of experiment.

The segment of the gastrointestinal tract under study was the esophagus. This study was conducted on the Wistar albino rats, who were divided into groups of 'Controls', namely A1, A2 & A3 where n= 10 for each group and exposed groups B, C & D where n=15 for each group. Each rat in the exposed group was given carbonated drinks for a duration of 1, 3 and 6 months along with regular food. The controls were fed water only, along with regular diet.

The exposed animals took to the consumption of cola drinks eagerly and the swishing sound that came from opening the bottle cap would alert them of the 'treat' waiting for them and they would gather around the feeding bottle in the anticipation of the fizzy drink.

Once dissected, at the end of the first month, their oesophagus were removed and preserved in 10% formalin, dehydrated in alcohol, cleared in xylene, embedded in paraffin, sectioned into 5µm thick sections by a microtome, stained in hematoxyline and eosin, observed under a microscope and micrometry was done.

Calculation of sample size was done using the open epi sample size calculator, with confidence interval of 95% & margin of error 5%⁷. Sample size was calculated to be 75 wistar rats.

Shapiro-Wilk test was applied to check the normal distribution of data. As p-value was more than 0.05, so there was normal distribution of data. Independent sample t-test was used to compare the microscopic parameter esophageal width between the control and exposed groups. Statistical software SPSS 20 was used to analyze the data with p-value significant at 0.05.

Results

On observation it was noted that the esophageal width was markedly increased amongst the exposed group compared to the controls, p-value <0.01. On comparison of the tissue edema it was observed that the exposed animal's esophageal tissue was markedly edematous with presence of inflammatory cells compared to the controls as in figure-1.

Subsequently in the third month exactly the same results were obtained with the same esophageal width and presence of edema as in figure-2. Lastly at the end of the sixth month the width remained the same as shown in figure 3.

Descriptive statistics was applied to analyze the mean width of esophagus in both the exposed (B, C ,D) and control groups (A1, A2, A3). Independent Sample t-test was done to compare the mean width of esophagus among groups B & A1, C & A2 and D & A3.

After a duration of one month, mean esophageal width in the Exposed Group B which was $14.60 \pm 4.43\mu\text{m}$ and mean width of esophagus in the Control Group A1 which was $6.2 \pm 1.87\mu\text{m}$ were compared using the independent sample t-test. Statistical significant difference in the mean esophageal width was observed among the two groups with p-value <0.01.

At the end of third month, mean width of esophagus in exposed group C and control group A2 were compared using t-test for independent samples. The mean esophageal width in the Exposed Group C was $9.40 \pm 2.35\mu\text{m}$ and mean width of esophagus in the Control Group A2 was calculated as $5.80 \pm 1.81\mu\text{m}$. There was significant mean difference with $p < 0.01$ between the two groups.

The rats, who were exposed to the carbonated drinks for a period of 6 months i.e. group D were dissected and their esophageal wall thickness was measured by micrometry along with the control group A3. Mean esophageal width in exposed group D $13.60 \pm 2.89\mu\text{m}$, when compared with the control group A3 $4.50 \pm 1.43\mu\text{m}$ was found to be statistically significant with p-value <0.01.

Table 1. Comparison of microscopic parameters between the exposed and control groups at the end of the first month.

GROUP	Mean	Standard Deviatio	p-value
Width (µm) Group B (n=15)	14.60	4.437	<0.01*
Group A1 (n=10)	6.20	1.874	

Table 2. Comparison of microscopic parameters between the exposed and control groups at the end of the third month

Group	Mean	StandardDeviation	p-value
Width(µm) Group C (n=15)	9.40	2.354	<0.01*
Group A2 (n=10)	5.80	1.814	

Table 3. Comparison of microscopic parameters between the exposed and control groups at the end of the sixth month.

GROUP	Mean	Standard Deviation	p-value
Width(µm) Group D (n=15)	13.60	2.898	<0.01*
Group A3 (n=10)	4.50	1.434	

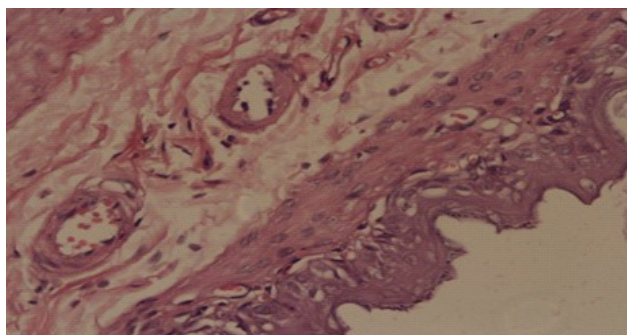


Fig.1. Control: H & E stained 5µm thick section of oesophagus showing mucosa & submucosa Of a control specimen of wistar rat at 40X.

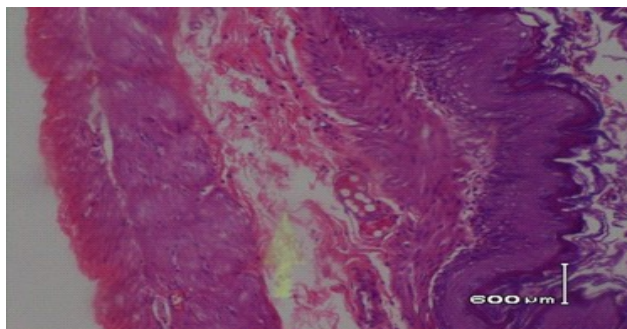


Fig.2. Exposed: H & E stained 5µm thick section of oesophagus showing haemorrhage and edema in a wistar rat on carbonated drinks at 40X.

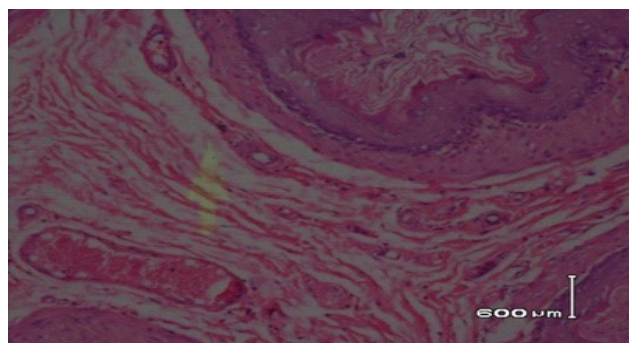


Fig.3. Exposed: H & E stained 5µm thick section of oesophagus showing inflammatory cells and edema (Shown by yellow arrow) in a wistar rat fed on carbonated drinks at 40X.

Discussion

The present experimental study was carried out on seventy five rats of wistar strain (gender nonspecific) which were divided into both control (A1, A2 & A3) and exposed groups (B, C & D). In this study, carbonated drink' consumption and its damaging effects on the esophageal mucosa of albino rats were examined in both control and exposed groups.

After dissection, visual examination showed esophageal discoloration in the retrieved samples of exposed groups at the end of the first, third and sixth months but was statistically insignificant.

JS Ren et al conducted a large prospective cohort study in United States and investigated the relationship of carbonated beverages with the upper GIT cancers. According to the results of his findings, trauma caused to the upper gastrointestinal mucosa was due to the varied nature of chemicals used in the carbonated drinks (phosphoric acid & citric acid)⁸. Adel et al also investigated the chronic effects of soft drink consumption on the health state of Wistar rats. They observed the microscopic features of the esophagus of the rats exposed to soft drinks and found resultant increase in the capillary permeability with resultant leakage of plasma proteins, arteriolar dilation that played a major role in the tissue hyperemia⁹. Waleska et al determined the effects of fructose present in sufficient amounts in soft drinks and demonstrated that high fructose intake promotes inflammation by accumulation of inflammatory substances such as TNF-alpha¹⁰. Another concordant study, conducted by Oliveira et al. noted histopathological changes

in the rats esophagus after exposing them to carbonated water and found inflammatory infiltrate predominantly diffuse mild to moderate and tissue edema in the exposed group¹.

The controls showed no such changes and their retrieved samples were unremarkable.

Esophageal edema was looked for on gross examination. Significant edema ($p < 0.04$) was noted on the samples recovered from the animals of C group (third month) as opposed to the controls i.e. A2. Carbonated beverages have ingredients that cause soft tissue inflammation. According to the findings of Ayesha et al, the epithelial changes, inflamed soft tissue and edema observed in experimental group, may be the result of constant irritation from carbonated beverages' acidic and fizzy nature^{11,12}.

Our study results demonstrated edema to be minimal in the animals dissected at the end of the first and sixth months.

Akash et al found that, in healthy people, drinking carbonated beverages increases the frequency of transient lower esophageal sphincter relaxation and reduces lower esophageal sphincter pressure. The contributing factors towards edema were the increase in vascular permeability, widening of tissue spaces caused by substance P release, histamine, leucotrienes, and bradikinin all initiating release of proteins and fluid into the surrounding tissue¹³. Lymphatic channels clear the leaked fluid and collected proteins from the edematous tissue but overwhelmingly large amounts of these fluids and collected proteins choke these channels giving rise to secondary inflammation and hyperplastic lymph follicles^{14,15}. Ultimately, through the above process the tissue becomes edematous¹⁹. The low molecular weight solutes and irregular movement of water between intravascular and interstitial spaces, the vascular hydrostatic pressure and plasma colloid osmotic pressure giving rise to increased capillary pressure allow fluid to escape into the interstitium, overwhelming the lymphatic outflow and cause edema to develop^{16,17}.

On gross examination, in the exposed group, esophageal haemorrhages were statistically significant ($p < 0.025$) in the first month but were insignificant in the subsequent third and sixth months.

Adel et al showed that the rise in MDA levels and decrease in the expression of antioxidants GR, GPx, and catalase in his study, drinking carbonated soft drinks on a regular basis caused oxidative stress in Wistar rats. Oxidative stress has been linked to the etiopathogenesis of a number of chronic diseases. Oxidative damage to cells, tissues, and organs is one of the consequences of uncontrolled oxidative stress, which occurs when antioxidant and prooxidant levels diverge in favor of prooxidants. High levels of free radicals or reactive oxygen species (ROS) have long been known to directly harm lipids¹⁸. An injury no matter how insignificant will cause an accumulation of edema with superimposed bacterial infection which may result in capillary bleeding and rupture resulting in hemorrhage¹⁹.

On microscopic examination, thickness of the esophageal wall was compared from samples retrieved in the first, third and sixth months. All the exposed groups showed an increase in the wall thickness on micrometry which was statistically significant ($p < 0.04$). The controls showed no such increase in wall thickness.

Accumulation of edema fluid, hemorrhage in the surrounding tissue and collection of inflammatory cells within mucosa and submucosa were responsible for the increase in the esophageal wall thickness²⁴. This finding is consistent with the study by Hamaguchi et al. who studied the expression of cytokines and molecules in rats with chronic esophagitis. The mRNA expression of various cytokines such as IL-1 & TNF-alpha and cell adhesion molecules as ICAM-1 was significantly higher in esophageal lesions than in normal esophagus of rat model²⁰.

Only to a limited extent can the mucus secreting cells of the esophagus protect the mucosa from the acid reflux¹¹. The regurgitation gives rise to reflux esophagitis, heart burn & mucosal erosion²¹. The acid rich gastric fluid regurgitation can

also compound the injury to the esophago-gastric mucosa and bring about inflammatory changes with migration of inflammatory cells, mast cells, lymphocytes to the site of injury, causing increased wall thickness¹⁵. Carbonated beverages prolong healing time^{22,23}. Hence, changes were most evident in the exposed group D.

Conclusion

The findings of this study suggest that prolonged consumption of carbonated beverages has deleterious effects on the esophagus. However, the underlying pathophysiology and its human implications require additional research.

Conflict of Interest

Authors have no conflict of interest and no grant/funding from any organization

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