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Histopathological and Biochemical Toxic Alterations in Heart Tissue of Rats Exposed to Conventional Cigarette

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ABSTRACT

The present experimental study was carried out to identify histopathological and biochemical alterations in the hearts of rats exposed to conventional cigarette vapour. The methods included the use of 50 well-acclimated (25 treated and 25 control) rats for the personnel and laboratory procedures. The rats were subjected for 40 days (twice a day) to conventional cigarette vapour. After the end of the experimental exposure, the rats were sacrificed to prepare myocardial tissue-based microscopic slides. The blood albumin and cholesterol levels of the rats exposed to cigarette smoke were found to be significantly lower ($p< 0.05$) than those of the control animals. Alkaline phosphatase (ALP) and aspartate aminotransferase concentrations increased significantly ($p<0.05$) in the smoking-treated group's liver enzyme profile (AST). When compared to that from the control animals, the level of lactate dehydrogenase (LDH), a marker of tissue damage, substantially ($p<0.05$) increased in the cigarette-smoke-exposed rats. The histopathological outcome demonstrated the presence of swelling of the myocardial fibres. The indications revealed the occurrence of some vacuoles within the heart tissues. In addition, there was an increase in the oedema-originated spaces between the myocardial fibres. Moreover, unambiguous evidence of blood vessel-related light congestion. Furthermore, the light microscopy displayed the clear presence of myocardial fibres with an irregular pattern. The results, unveiled here, may indicate some dangerous effects of the vapour produced by conventional smoking on the myocardial tissues of Lab rats as indicated by the changes in the biochemical parameters and histopathological features.

Introduction

Tobacco smoking is inhaling vapour from combusted tobacco through the mouth and, more commonly, the lungs. Cigarettes are by far the most regularly consumed substance, however, cigars, cigarillos, pipes, and even water pipes are occasionally used. Some regions of the globe also see significant demand for "smokeless" tobacco products (1). Tobacco products are used, and the chewing tobacco is either inserted between the cheeks and gums or inhaled via the nostrils via the same previous method. Like cigarettes, smokeless tobacco usage carries serious health hazards (2,3).

Heart failure (HF) affects over 6.5 million Americans, with a new case being diagnosed every year at a rate of 960,000. The estimated number of Americans with HF is projected to rise by 46% from 2012 to 2030, although incidence rates are likely to remain the same over that time (4,5). Some researchers believe that the widespread application of medical therapy based on established guidelines is to blame for the rise in the incidence of heart failure with reduced ejection fraction

(HFrEF) (6–8). Likewise, the ageing generation and the increasing incidence of comorbidities, especially obesity and diabetes, will probably lead to an increase in the incidence of HF with preserved ejection fraction (HFpEF) in years. The continuous use of effective medications and the management of risk factors is crucial if we are to lessen the impact of HF. As stated, cigarette smoking is a major contributor to the development of cardiovascular disease (CVD) and is responsible for about 40% of all CVD-related fatalities. Smoking is linked to a 60% increased risk of HF, according to a systematic review and meta-analysis published in 2015 (9,10).

Nicotine-based smoke may contribute to an increased incidence of CVD. The vast majority of the negative health effects of cigarette smoking come from the smoke's other chemicals. Proof for clinically substantial abnormalities in cognitional or emotional situations in adult smokers as adolescents and later quit is lacking, despite claims to the contrary based on research with other species. Children and adults alike are at serious risk when exposed to secondhand smoke. As a result, nonsmokers who are subjected to secondhand smoke are more likely to develop cancer, cardiovascular disease, and respiratory illness (3).

The present experimental study was carried out to identify histopathological and biochemical alterations in the hearts of rats exposed to conventional cigarette vapour.

Materials and methods

Ethics

The experiment and treatments of the laboratory animals were done following ethical and legal procedures from national and international guidelines that focus on animal care and use.

Design of experiment and tissue slide preparation

The experiment was completed in a period between July 10, to September 10, 2021, at the College of Medicine, Waist University. The methods included the use of 50 well-acclimated (25 treated and 25 control) Wistar-rats (mean weight was170gms). All the animals spent their time in sanitary cages of 33× 20.5×19cm, which were kept in a typical environment with regular lighting and ventilation (light/dark cycle of 12 hours each, temperature of 28 to 31 degrees Celsius, and humidity of 50 to 55 %).

At the time of smoking exposure, a locally-modified anaerobic box chamber (25.9cm×23.4cm×20.9cm), similar to that from Montanari and Christian (11), was used to deliver conventional cigarette smoking into the chambers.

The rats were subjected to 40 days (once a day of 10 smoking cigarettes for one hour) of conventional cigarette vapour directed toward the animals using a specific motor. The control animals received no smoking throughout the experiment period. After the end of the experimental exposure, the rats were chloroform-anaesthetized and sacrificed by separating the neck. The blood and heart specimens were collected from all animals for examining biochemical and histological changes. The first and most important step is to obtain and store the specimens properly. Cardiac tissue harm can be avoided in rodents with prompt removal and cooling of the heart. For that, the cardiac samples were collected and cooled immediately to prevent any damage to the tissues. The histopathological slides were generated according to Disbrey and Rack (12).

For the identification of the serum levels of biochemical parameters, a spectrophotometric technique was followed as mentioned by Huang et al (13). Spectrophotometric procedures were used for the determination of the serum level of some parameters of hepatic diseases. The liver parameter in serum may be measured quickly using spectrophotometry in a short-interval enzyme-activity analyzer. The device is simply a spectrophotometer with a variety of different wavelengths. The test cuvette's altered absorbance is measured in comparison to water. With the help of this method, it was feasible to calculate the reaction's initial velocity, which was inversely

related to the enzyme concentration. Several materials, including rat serum, were utilized to test the enzyme activity using this method (13).

Statistical processes

The informational data were SPSS-analyzed and displayed, using a *t*-test, and mean ± standard deviation (SD) for displaying of analyzed data. A significant process was determined if *p* is less than 5%.

Results

Biochemical data

The blood albumin and cholesterol levels of the rats exposed to cigarette smoke were found to be significantly lower ($p<0.05$) than those of the control animals. The amounts of the ALP and AST, previously known as GOT, in the smoking-treated group's liver enzyme profile showed a significant ($p<0.05$) increase. For the indication of tissue damage, the LDH significantly ($p<0.05$) increased in the cigarette-smoke-exposed rats when compared with that from the control animals (Figure 1 and table 1).

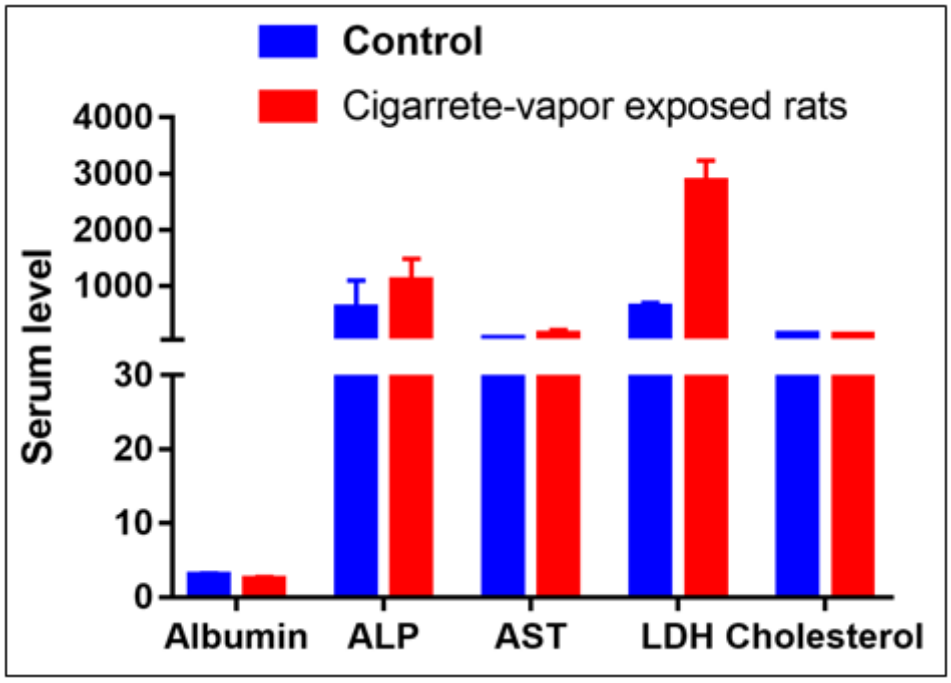

Table 1: Blood biochemical parameters in conventional smoking-exposed rats.

Group Statistics (N=25/each)				
Parameters	**Case**	**Mean**	**SD**	***P* value**
Albumin	**Normal**	3.0182	0.19242	0.0001*
	Cigarette	2.4809	0.24257	
ALP	**Normal**	609.2750	487.50385	0.023*
	Cigarette	1096.8500	386.00144	
LDH	**Normal**	620.40	77.775	0.00001*
	Cigarette	2855.30	379.144	
GOT	**Normal**	65.8945	14.47874	0.002*
	Cigarette	149.5070	70.26729	
GPT	**Normal**	78.1510	6.04335	0.559**

	Cigarette	83.7000	28.85955	
Cholesterol	**Normal**	141.80	13.927	0.0001*
	Cigarette	121.20	6.408	

*Significant $p< 0.05$, **Non-significant $p> 0.05$.

Histopathological alterations

The histopathological outcome demonstrated the presence of swelling of the myocardial fibres. The indications revealed the occurrence of some vacuoles within the heart tissues. In addition, there were increases in the oedema-originated spaces between the myocardial fibres. Moreover, there was clear evidence of blood vessel-related light congestion. Furthermore, the light microscopy displayed the clear presence of myocardial fibres with irregular patterns (Figure 2).

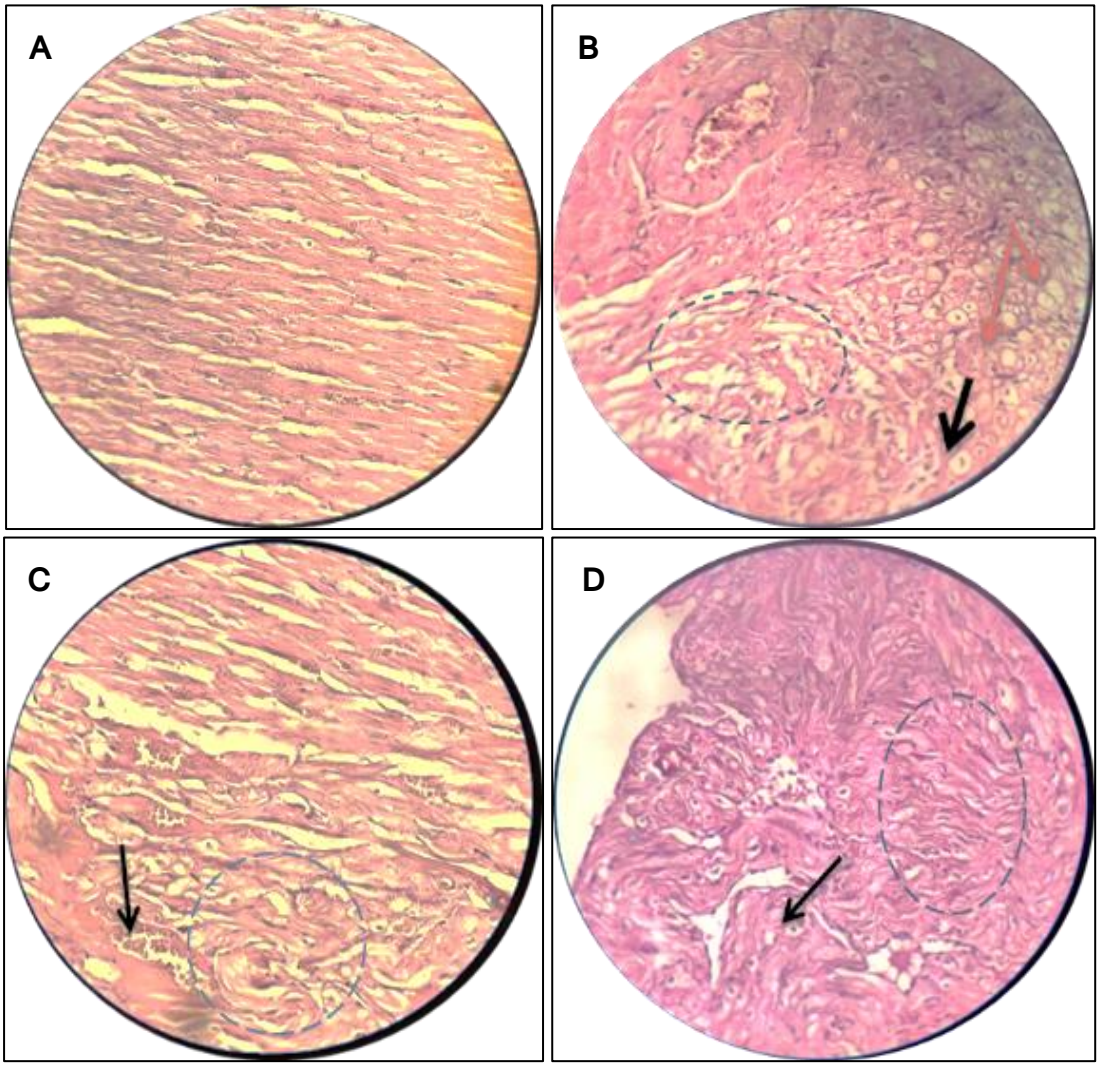

Figure 2: Heart-histopathological features of control and conventional smoking exposed rats. **A**: Shows the normal tissue (control group). **B**, **C**, and **D**: Demonstrate the tissue histopathological changes of conventional smoking exposed rats; **B**: Edema-originated spaces between the myocardial fibers (**Blue circle**), swelling of the myocardial fibers (**Black arrow**) (**B**&**D**), and vacuoles (**Red arrow**). **C**: Blood vessel related light congestions (black arrow) and myocardial fibers with irregular pattern (**Blue circle**) (**C**&**D**). H&E 40X.

Discussion

Cigarette smoking is a commonly documented standalone risk factor for ischemia and atherosclerosis of the heart and CVD. Exposure to passive smoking can also cause increase the risk percentage of critical health problems. Endothelial-based dysfunctional processes, intimal hyperplasia, and arterial wall-related thickening in the human heart and kidneys have all been linked to cigarette smoking. Histological images of myocardial tissue from rats subjected to smoking vapour indicate changes consistent with the findings of our rat models, in which a daily amount of cigarette vapour comparable to that breathed by a human smoker was delivered to these rats for only 60 mins per day (14,15).

For this reason, we investigated the histopathological and biochemical effects of smoking a standard cigar on the hearts of rats. In rats subjected to traditional smoking vapour, the cardiac organ showed minor oedema and infrequent absence of myocardial fibre, as well as an elevation in the compression of heart muscle on the arteries, thus decreasing the diameter of these arteries and subsequently the smooth stream of blood through these vessels. These results agree with those of Adedayo et al. (16), who similarly noted the development of moderate oedema and momentary absence in these fibers. Nicotine can reduce coronary blood circulation, negatively impacting the individual with angina, as shown by (17), who observed that nicotine induces vascular narrowing. Mild dissociation between myocardial fibres was seen, as mentioned by Asadi *et al* (18), which could produce a detrimental effect on the myocardial muscle's ability to pump blood productively to body organs. Nevertheless, the decreased performance may indicate cardiac reserve depletion (19).

The structural alterations directly linked with both free radical and cyanide harmful effects, all of these are critical constituents of smoke, and the present study confirmed this finding by showing that nicotine had a toxic impact on the elasticity of myocardial fibres due to the presence of an irregular pattern of these fibres (20). The Increases in tissue destruction could be due to increases in the production of oxygen free radicals or lytic enzymes. Moderate oedema and infrequent myocardial fibre damage were also seen by Adekomi *et al*. (21). This research also found that disturbances in heart function were associated with the swelling of cardiac cells and the presence of vacuoles. The myocyte hypertrophy is consistent with those obtained by other researchers, such as Danilo *et al* (19). Constriction of blood vessels was also observed in the current study, which is consistent with findings from a previous study by Al-Awaida *et al* (20) that observed detachment between myocardial fibres, invasion of lymphocytes, and congestion of the heart blood vessels of their waterpipe-exposed rats.

The results, unveiled here, may indicate some dangerous effects of the vapour produced by conventional smoking on the myocardial tissues of rats as indicated by the changes in the biochemical parameters and histopathological features.

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