



Protective Effects of Curcumin against Nicotine-Induced Damage on Esophagus Tissue in the Wister Rat

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ABSTRACT

Curcumin has been used in folk medicine for thousands of years as a food and traditional medicine. It is renowned for its antibacterial and antioxidant properties. This study assesses the potential impact of waterpipe smoking on the esophageal tissues of Wister rats (*Rattus norvegicus*). Furthermore, it aimed to investigate curcumin's efficacy in reducing cytotoxicity induced by tobacco smoking. A total of 27 Wister male adult rats were separated into three groups first acted as a control, the second group smoked, and the third group received daily intraperitoneal injections of 40 mg/kg of curcumin. Additionally, the rats were exposed to waterpipe smoking using a smoking machine for 30 days before dissecting the rat; serum samples were collected to measure enzyme activity. The esophagus was then isolated and prepared for light microscopy analysis. According to the findings of this study, it has been demonstrated that curcumin effectively decreased the activity of liver enzymes (AST, ALT, and LDH) which reduced the harmful effects of waterpipe smoking in rats. Treatment of curcumin with exposure to WPS showed a reduction in the thickness of the keratin layer with less inflammation in comparison to the waterpipe only; the results indicate that curcumin has a protective impact.

Keywords: Curcumin, Waterpipe smoking, Antioxidants, Histopathology

Introduction

The consumption of tobacco leads to several deadly illnesses, such as cardiovascular diseases, chronic obstructive pulmonary disease, and lung cancer, all of which are considered major health concerns worldwide.¹

It is estimated that tobacco smoke caused approximately one hundred million fatalities globally in the last century.² Research suggests that tobacco contains over 400 chemical compounds, several of which are toxic and carcinogenic.³ There is a belief that cigarette smoking's chemical agent, nicotine, goes beyond addiction behavior, and other chemicals contribute to smoking-related illnesses. The liver metabolizes nicotine, which can affect the balance between oxidants and antioxidants. Nicotine stimulates the production of free radicals and ROS, leading to oxidative stress and potentially damaging the antioxidant defense system.⁴

Polyphenols like curcumin (diferuloylmethane) have anti-inflammatory and antioxidant properties that help prevent toxin-mediated stress responses. They can also induce the expression of cytoprotective proteins, providing further protection.⁵

There is a potential for curcumin to act as an antioxidant by neutralizing nitric oxide and promoting the continuity of antioxidant enzymes like catalase and superoxide dismutase. It also can decrease the lipid peroxidation. Moreover, Nicotine has the potential to cause oxidative harm to tissues and organs, and however, curcumin is known to provide protection against such damage.⁶

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This study aims to analyze the histological and biochemical changes in rat tissues caused by sub-chronic waterpipe smoking. Specifically, the esophagus tissues were examined under a light microscope. Additionally, the potential protective effects of natural antioxidants, such as curcumin, were evaluated to prevent damage to the rat esophagus caused by waterpipe smoking.

Materials and Methods

Twenty-seven male Wister rats (*Rattus norvegicus*) that weighed between 150-180 g and were 6-8 weeks old were obtained from animal Houses at the Jordan University of Science and Technology Irbid, Jordan (September, 2020). The institutional research ethics committee from the Faculty of Allied Medical Sciences at Jadara University approved the study protocol and animal care procedures (MLS-R-6/1/2020). A total of 27 rats were divided into three groups (with nine rats in each group) for the study. The first group was exposed only to fresh air and served as the negative control, while the second group was exposed to flavored waterpipe smoke resulting from burning 20 g of moassal for 30 days. Group three was exposed to waterpipe and at the same time treated with (40 mg/kg/day) curcumin for one month.

Preparation of curcumin

Curcumin was purchased from (Sigma-Aldrich, China) and prepared using phosphate-buffered saline (pH 7.2, 0.1 M). The dose of curcumin was 40 mg/kg/day; the solutions were contained within 1 mL disposable insulin syringes.⁶

The Digital Smoking Machine

Shraideh *et al.*'s digital smoking device was utilized. This apparatus is suitable for subjecting rats to waterpipe /cigarette smoke.⁷

Protocol of Light Microscopy

The tissues from the esophagus were carefully removed, thoroughly washed with normal saline (0.9% NaCl), and then preserved in 10% saline-buffered formalin for at least 24 hours. Tissues were obtained after overnight recovery from the last smoke exposure to ensure adequate fixation. Rats were sacrificed by ether anesthesia. Dehydration

was achieved by passing tissues through a graded series of alcohol (70%, 80%, 95%, 100%, and 100%). After undergoing two rounds of xylene changes, the tissues were infiltrated with paraffin wax and embedded in pure paraffin wax. Using a Spencer 50 microtome, thin 5µm sections were obtained. These sections were then mounted on glass slides and stained with hematoxylin and eosin before being examined and photographed with a Zeiss photomicroscope. The photomicrographs were taken with a Moticam 2300 digital camera that had a resolution of 3.0 megapixels.⁷

Biochemical analysis

Tests including (ALT, AST, and LDH) were performed to evaluate enzyme activity; we bought ALT and AST kits from Teco Diagnostics, located in Anaheim, CA, USA. These tests were performed using a UV/VIS single-beam spectrophotometer (EMC-11D-V; EMCLAB equipment, Duisburg, Germany).⁶

The activity of the LDH and SGPT enzymes in the serum was evaluated by measuring their ability to oxidize NADH to NAD⁺ spectrophotometrically at 340. While the activity of SGOT enzyme in serum was calculated through its ability to reduce NADH to NAD⁺ spectrophotometrically at 340 nm.⁶

Statistical Analysis

Statistical Software Package for the Social Sciences (SPSS version 20) was utilized to analyze the data. All data for the treatment groups for each test were analyzed by one-way ANOVA considering results with P-value <0.05 as significant, the graphs were represented as mean ± standard error of the mean.

Results and Discussion

The Effect of Curcumin waterpipe smoking on liver Enzymes of Rats Exposed to Sub-Chronic Smoking

Table 1 shows the statistical analysis of three serum enzymes activity (AST, LDH, and ALT level) following sub-chronic exposure to waterpipe smoking with treatment by Curcumin. The treatment shows a significant effect on waterpipe smoking. The level of AST in the control group was 30.3 ± 0.112U/ L and significantly increased

following exposure to waterpipe smoking group 90.12±0.050 Treatment with curcumin decreased the level of AST significantly in the waterpipe smoking group (85.8±0.212) U/L. p= 0.01.

The treatment shows a significant effect on waterpipe smoking. The level of ALT in the control group was 35.5±0.260U/L and significantly increased following exposure to waterpipe smoking (51.61±0.250, p=0.0); treatment with curcumin decreased the level of ALT significantly in the waterpipe smoking group (38.24±0.110, p=0.01).

The level of LDH in the control group was 449.0±1.10 U/ L, which significantly increased following exposure to waterpipe smoking (676.0±0.50, p= 0.0); treatment with curcumin decreased the level of LDH significantly in waterpipe smoking group (280.0±0.57, p=0.0).

The Effects of Sub-Chronic Smoking on the esophagus sections

The esophagus Sections of the control group showed histologically normal esophagus tissue displaying standard histological structure; normal mucosa, submucosa, lamina propria, stratified epithelium cells, muscularis externa, and small mucous glands Figure 1. In contrast, the histological sections of water pipe smoking of the Esophagus tissue showed a thick keratinized layer (bold arrow), necrosis of some epithelial cells (thin arrow), and congestion of blood (star) Figure 2. The Esophagus tissue of rats treated with curcumin during waterpipe smoking Showed less thickness in the keratin layer (bold arrow) and infiltration of mixed inflammatory cells in Laminapropria (thin arrow) Figure 3.

This research was undertaken due to the growing global incidence of smoking and its related hazards. Various illnesses, including chronic obstructive pulmonary disease, cardiovascular diseases, and lung cancer, have been linked to smoking. It is crucial to conduct a thorough investigation into the harmful impact of tobacco on histological level and to explore the potential benefits of incorporating natural antioxidant-rich products like curcumin to alleviate its adverse effects. Smoking can severely damage cardiac tissue and increase the likelihood of heart attacks.⁸ Research has shown a notable dysfunction in the dilation of blood vessels in the heart, ultimately resulting in atherosclerosis. Additionally, respiratory sinus arrhythmia (RSA) - the natural fluctuation of heart rate during breathing - can interfere with parasympathetic effects.⁹

Table 1: Effect of Curcumin AST, ALT, and LDH level in rats exposed to waterpipe smoking

Animal groups	AST (U/L)	P- value	LDH (U/L)	P- value	ALT (U/L)	P- value
Control (fresh air)	30.3 ± 0.112	1.00	449.0 ± 1.10	1.00	35.5 ± 0.260	0.00
Waterpipe	90.12 ± 0.050	0.00*b	676.0 ± 0.50	0.00*b	51.61 ± 0.250	0.00*b
Waterpipe + curcumin	85.8 ± 0.212	0.00*b	280.0 ± 0.57	0.00*b	38.24 ± 0.110	0.01*b

*Mean significant P<0.05. a: compared to the control group, b to the waterpipe group.

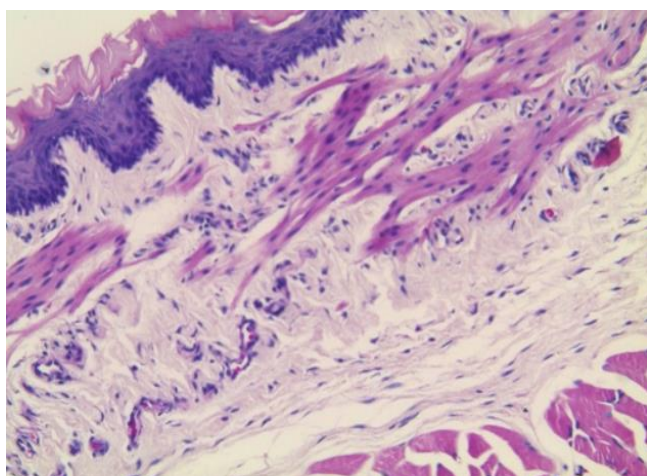


Figure 1: The esophagus of the control group showed normal tissue architectures: (Bar = 100µm). (H&E)

Esophageal cancer is commonly associated with smoking as a significant risk factor.¹⁰ The study results indicate that smoking has a negative impact on the histology of the esophagus. There is an inflammatory response and thickening of keratin, as well as the presence of necrotic cells. After being treated with curcumin during waterpipe smoking, the Esophagus tissue of rats exhibited a decreased thickness in the keratin layer and a reduction in the infiltration of mixed inflammatory cells in Laminapropria.

According to our findings, after extended hookah smoking, there was a significant increase in important serum enzymes ALT, LDH, and AST compared to the control group. (P<0.05). According to research, rats exposed to smoking experienced increased activity of liver enzymes, indicating liver damage. Toxic chemicals released through smoking harm liver cells and result in the production of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6.¹¹ Curcumin is known as a powerful antioxidant and anti-inflammatory agent,¹² It contains polyphenols (diferuloylmethane), which reduce toxin-mediated stress responses through both its antioxidant and anti-inflammatory properties.^{13,14}

We conducted a study to explore how curcumin can protect esophagus tissues and prevent the adverse effects of smoking on liver enzymes ALT, LDH, and AST levels. Compared to the normal group, the

smoking group experienced a significant increase in serum ALT, AST, and LDH enzymes. However, the group that received curcumin treatment showed decreased enzyme activity, indicating a protective effect of curcumin against smoking-induced toxicity. According to another study, administering curcumin to rats exposed to smoke reduced serum enzyme levels. (AST, ALT, and LDH).¹⁵

Conclusion

The research has confirmed that smoking waterpipe tobacco is linked to adverse health effects. Findings indicate that curcumin reduces the hepatotoxicity induced by smoking and can provide some protection against the harmful effects of this type of smoking.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

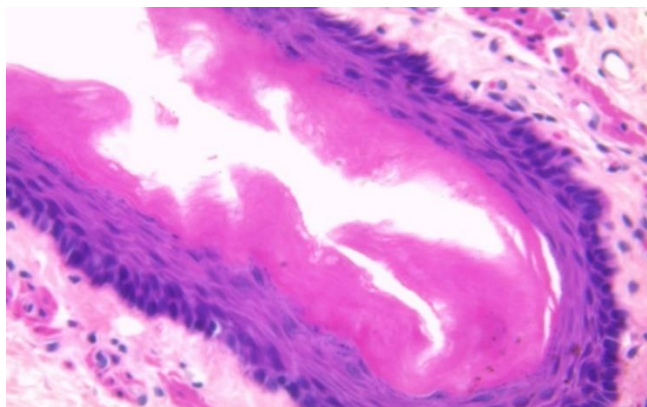


Figure 2: Esophagus of rat exposed to Waterpipe smoke (Bar = 100µm). (H&E)

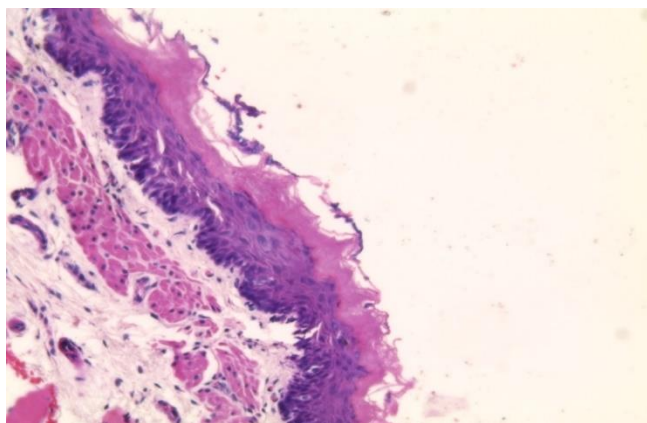


Figure 3: Esophagus of rat exposed to waterpipe smoking and treatment by curcumin (Bar = 100µm). (H&E)

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