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RESEARCH ARTICLE

The Effects of Garlic Treatment on p53 Expression in the Heart Ventricle of Smoking Rats: A Passive Smoking Model of Cigarette Smoke and Waterpipe Smoke

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ABSTRACT

The present study was conducted to examine the effects of smoking (cigarette or waterpipe) on the heart ventricle using several approaches including histological and immunohistochemical staining.

The study was carried out on 24 male albino rats which were randomly assigned into 3 groups (n=8 per group). Group (1) was negative control exposed only to fresh air; groups 2 exposed to the most used cigarette brands in the Jordanian market (red LM cigarettes) as 1 cigarette/rat/day for 30 consecutive days, and groups (3) was exposed to waterpipe smoke. Both groups were treated with the garlic extract. Rats in group (3) were exposed to flavored waterpipe smoke resulting from the complete burning of 20 g from one of moassal for a period of 30 days one session a day.

Histological studies showed that the exposure to smoking has been associated with adverse effects in the tissues. Treatment with garlic extract restored most of histological changes. Both types of smoking models induced the expression of p53 protein in the heart ventricle. However, p53 was slightly expressed in the heart.

Smoking effects were studied on the heart ventricle using histology and immunohistochemistry. Histological changes of the heart ventricle, and the effects the expression of p53 expression were observed. Treatment with the garlic extract showed protective effects against adverse effects of smoking models.

INTRODUCTION

Passive smoking has been shown to negatively affect people's health who were exposed to second-hand smoke (He et al., 2020). Unfortunately, children are the most affected passive smokers as the family members who smoke at home exposed them to such environment (An et al., 2021). By law, the government has imposed a campaign to prohibit smoking, but a common mode of smoking was ignored, and it's the waterpipe or shisha smoking (Nurhasana et al., .2020). Convenience, affordability, and presumed less harmfulness of shisha smoking leads to an increasing number of

women and children that smoke at home, thus exposing the entire household to the danger of passive smoking (Starker et al., .2022). However, in the pathologies associated with smoking, oxidative stress is among the most important mechanisms that mediate the injurious effects of tobacco smoke (Prakash et al., 2022).

p53 is a protein that plays a crucial role in maintaining the stability of the genome and is also known as "the guardian of the genome" (Zeng et al., 2022). It is a versatile protein involved in various cellular activities ranging from DNA repair to apoptosis, but the most important functions of p53 are as a transcription factor regulating cell cycle arrest, apoptosis, and DNA repair (Song et al.2022). There are several agents that are known to cause oxidative damage that can lead to p53 activation, and some of these agents are cigarette smoke and waterpipe smoke (Seo et al., 2023).

ROLE OF P53 IN CARDIOVASCULAR HEALTH AND DISEASE

The tumor suppressor p53 protein and acute inflammation both contribute to cell apoptosis (Zhu et al., 2023). After cardiac ischemia/reperfusion injury, p53 protein expression increases and reaches a peak at about 6 h post-injury (Li et al.2021). Activation of p53 leads to increased expression of pro-apoptotic genes such as Bax and p21 and causes mitochondrial dysfunction that activates the caspase-dependent apoptotic signaling pathway (Zhan et al., .2022). p53 exerts a protective effect against myocardial ischemic injury by mediating the expression of the anti-apoptotic Bcl-2 protein (Yang et al., .2023). One of the heart ventricles, p53-dependent apoptotic pathways may be mediated by c-jun-N-terminal protein kinase (JNK) (Li et al., 2022).

p53 expression increases in the heart after myocardial infarction, and p53 activity increases in the myocardium before visible infarct development (Ikeda et al.,.2021). The tumor suppressor p53 protein is required for induction of the G1 transient cell cycle arrest that occurs during the acute phase of irreversible myocardial injury (Liu et al.,.2022). Activation of the apoptotic death program is a common consequence of activating signaling pathways controlled by anti-apoptotic Bcl-2 family proteins (Guo et al.,.2024). Inhibition of JNK activity prevents apoptosis in such cells, suggesting that p53 activation may promote resistance to stresses by attenuating the signaling pathways leading to cell death (Zhu et al., 2023).

GARLIC AS A POTENTIAL THERAPEUTIC AGENT

Garlic (Allium sativum L.), a widely cultivated plant globally, has historically served as both a seasoning and a medicine in various cultures and medicinal traditions (Abdel-Baky and Abdel-Rahman, 2020). Its health-promoting properties have been linked to a spectrum of organ protection, including cardiovascular, nephrocyte, and retinopathy (Valls et al., 2022). Increased levels of pro-inflammatory cytokines and the production of reactive oxygen species (ROS) are involved in the pathogenesis of various diseases (Asgharpour et al., 2021). Garlic extract and its components, including the organosulfur-containing compounds allicin, diallyl sulfide, diallyl disulfide, and S-allyl cysteine, have shown the capability to inhibit the generation of oxidative damage and lipid peroxidation (Jeremic et al., 2020). Numerous studies have suggested that garlic has antioxidant properties (Ansary et al., 2020). Garlic and its sulfur-containing compounds have exhibited the ability to neutralize or scavenge free radicals in laboratory studies, which may provide therapeutic benefits for some diseases (Recinella et al.2023).

Information from various studies suggests that garlic extracts may serve as novel approaches for oxidative damage modulation (Alrumaihi., 2020). Garlic beneficially affected oxidative damage and antioxidant defense systems in the organs of rats with doxorubicin-induced cardiotoxicity (Sánchez-Gloria et al., 2022). Dietary supplementation with garlic decreased blood glucose and glycation end products, advanced glycation end products, malondialdehyde, and total nitric oxide (Pathak et al., 2020). In contrast, designer zwitterionic polysaccharides from garlic were able to scavenge hydroxyl

and DPPH-type free radicals and showed good antioxidant properties (Bedouhene et al., 2024; Das et al., 2024).

Exposure to cigarette smoke increases comorbid health problems, such as respiratory and cardiovascular diseases (Mallah et al., 2023). Passive smoke exposure (PSE) causes adverse effects on various organs, including the hearts, lungs, and brains, of non-smokers (Cha et al., 2023). PSE effects on cardiovascular disease, including vascular and cardiac effects, were reported to be like those in active smoking (Arechavala., 2021). In rats, PSE promoted exaggerated vasoconstriction and increased cardiac TNF-α expression, which synergistically contributed to coronary atherosclerosis progression (Espinoza-Derout et al., 2022). Even at low doses of smoke, the adverse effects on the cardiovascular system and organs were most sensitive (Costa-Beber and Guma, 2022). Exposure levels equivalent to exposure of 5 cigarettes were found to have comparable impacts on the brain as 17 active smoking cigarettes, indicating that the dose of smoke uptake may result in an underestimation of PS risk to the brain and cardiovascular system in epidemiological studies (Salehi et al.2023).

In this regard, the current study aimed to investigate p53 expression in the hearts of smoking rats, using exposure to cigarette smoke and waterpipe smoke as passive smoking models. Moreover, the effects of garlic treatment on p53 expression and related mechanisms were evaluated.

METHODOLOGY

Experimental Design

This experimental study was carried out on 24 male albino rats. Male albino rats (Rattus ratas) weighted 50-180 g (6-8 weeks old) were obtained from animal house at the University of Science and Technology and maintained under optimal conditions of diet and temperature.

Rats were randomly assigned to one of 3 groups (n= 8 per group), group (1) was control group exposed only to fresh air, group (2) exposed to the most used cigarette brands in the Jordanian market (red LM cigarettes) as 1 cigarette/rat/day for 30 consecutive days. Rats in group (3) were exposed to flavored waterpipe smoke resulting from the complete burning of 20 g from one of moassal for a period of 30 days one session a day. At the end of the experiment, histological and imunohistochemical studies were performed. Cigarette smoking and waterpipe smoke rats were treated with (100 mg/kg) garlic extract for one month at the same time of smoke exposure.

Preparation of Garlic Extract

Garlic extract was prepared according to Patricia Prati, et al. (2014) procedure; the raw garlic bulbs were manually peeled after immersion in water for 30 minutes at room temperature. Garlic was then processed into paste by using a max machine stainless- steel cutter. Garlic bulbs were crushed using a stainless-steel industrial blender and subsequently mixed with 50% of water (based on the garlic weight) to help homogenization. The paste freshness color was preserved by the addition of 0.1% potassium sorbate and 2% citric acid (dry), respectively, based on the total mass. Preliminary assays were carried out to determine the ideal amount of citric acid, and the concentration necessary for the paste to reach pH< 4.0, helping to maintain the natural light color of the product. After processing, the garlic products obtained were placed in 200 g-plastic containers and stored at controlled temperature of 25°C± 2°C, simulating room temperature. This extract is rich in active gradient allicin which considered as potent antioxidants (Patricia Prati, et al., 2014).

The digital smoking machine

The digital smoking apparatus designed by Shraideh, et al. (2011) was used. This apparatus is suitable for the exposure of rats to waterpipe /cigarette smoke (Shraideh, et al., 2011).

Routine histological staining and indirect immunoperoxidase staining were carried out.

RESULTS OF HISTOLOGICAL STAINING AND IMMUNOHISTOCHEMICAL FINDINGS

Histological findings of ventricles of the heart in study groups

Result of control group

Photomicrographs of control sections showed normal cardiac muscle fibers (Figure 1).

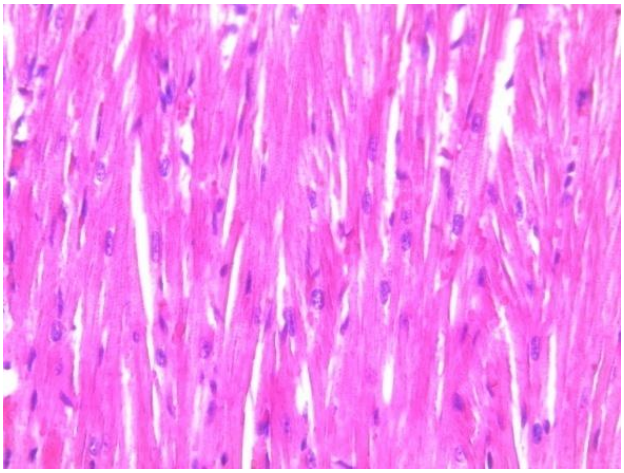

Figure 1: Normal heart ventricular tissue. Normal cardiac muscle fibers (M) and intercalated disc (arrow). H&E stain. 400X.

Result of exposure to cigarette smoke

Heart ventricular tissue of this group had shown some degree of separation and disruption between cardiac muscle fibers, the size of muscle fibers is decreased and spaces between them were widened, congested capillaries, and some inflammatory cells were seen (figure 2).

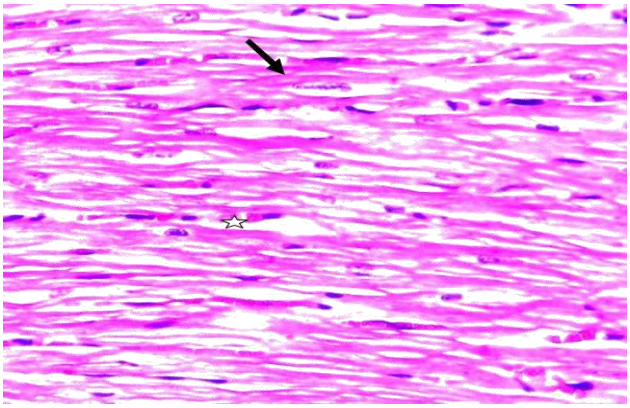

Figure 2: Ventricle of the heart of a rat exposed to cigarette smoke. Cardiac muscle fiber (Arrow). H&E stain. 400X.

Result of exposure to waterpipe smoke

Heart ventricular tissue of this group had also shown lymphocytic infiltration, with some degree of separation between cardiac muscle fibers and chromatin condensation (Figure 3).

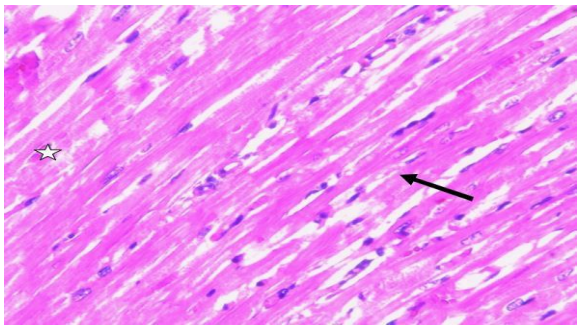

Figure 3: Ventricle of the heart of a rat exposed to waterpipe smoke. Cardiac muscle fiber (Arrows) and H&E stain. 400X.

The impact of treatment with garlic extracts on ventricle of the heart of rats exposed to cigarette smoke

As demonstrated in Figure (4), garlic extract showed no protective effects, still disruption of muscle fibers.

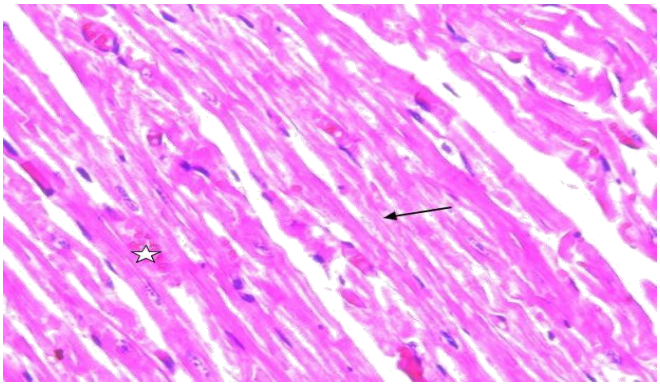

Figure 4: Ventricle of the heart of a rat exposed to cigarette smoke and treated with garlic extract. Bleeding (Star). Cardiac muscle fiber (arrow). H&E stain. 400X.

The impact of treatment with garlic extracts on ventricle of the heart of rats exposed to waterpipe smoke

As demonstrated in figure (5), garlic extract showed partial protection effects against waterpipe smoke adverse effects, the intercalated disc is present with uniform nuclei.

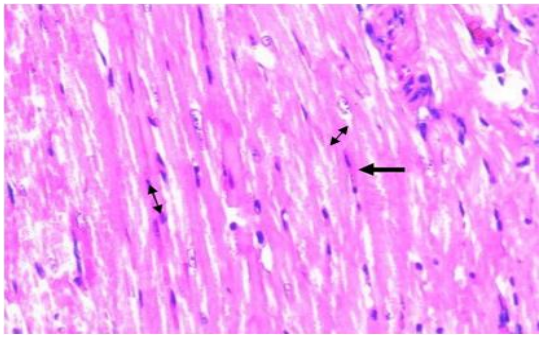

Figure 5: Ventricle of the heart of rat exposed to waterpipe smoke and treated with garlic extract. Cardiac muscle fibers (arrows) and intercalated disc (two heads arrow). H&E stain. 400X.

Results of Immunohistochemistry studies

The expression of p53 in the control tissues of the heart ventricle

Under physiological conditions, expression of p53 in heart ventricle exhibited some expression of p53 (Figure 6).

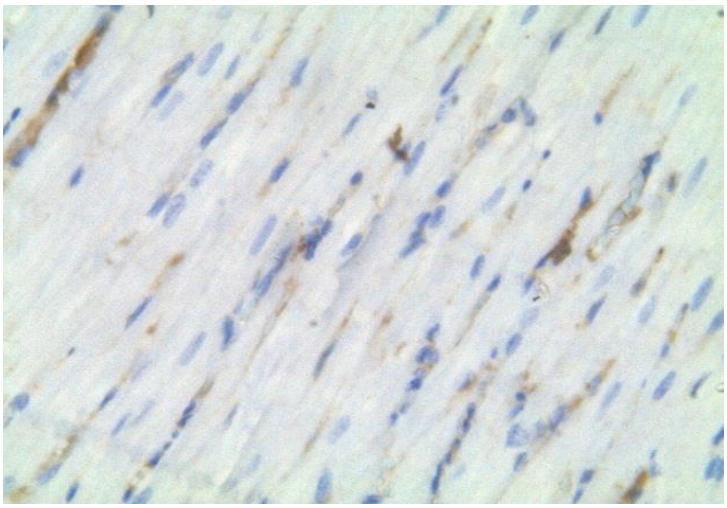

Figure 6: Expression of p53 in heart ventricle. Expression of p53 (brown color). 400X.

Expression of p53 in cigarette smoking tissues of the heart ventricle

As demonstrated in figure (7), there was an induced expression of p53 due to the exposure to cigarette smoking in the heart ventricle. This expression was observed in the nucleus.

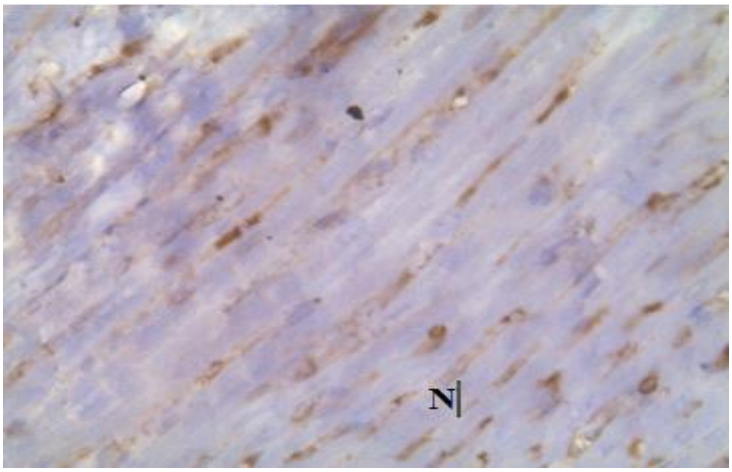

Figure 7: Expression of p53 in the heart ventricle of cigarette smoking exposed rat. The expression was observed in the nucleus (N). 400X.

Expression of p53 in waterpipe smoking tissues of the heart ventricle

As seen in figure (8), the exposure to waterpipe smoking induced the expression of p53 in the heart ventricle rats.

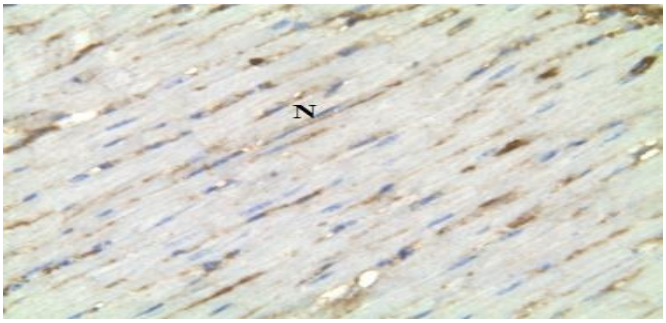

Figure 8: Expression of p53 in the heart ventricle of waterpipe smoking exposed rat, the reexpression was observed in the nucleus of the heart ventricle (N). 400X.

The Effect of Treatment with Garlic on expression of p53 in the Heart Ventricle of Rats Exposed to Cigarette Smoking

As illustrated in figure (9), the treatment of garlic for smoking group did not lower expression of p53 in heart ventricle, and it seems that expression of p53 exceeded the cardiomcytes and reached other stromal components of the ventricle including blood vessels.

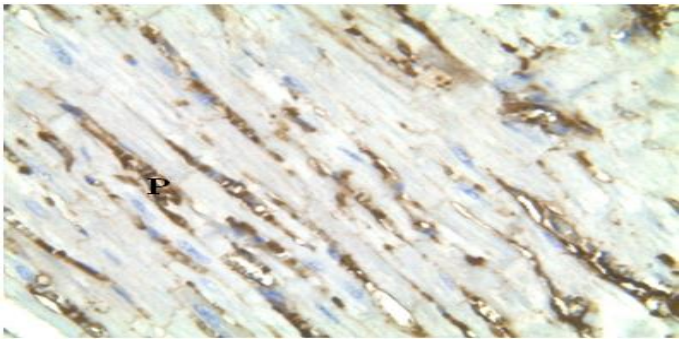

Figure 9: The effect of treatment with garlic on expression of p53 in the heart ventricle of cigarette smoking exposed rat. The expression of p53 exists (brown color), 400X.

The effect of treatment with garlic extracts on expression of p53 in the heart ventricle of rats exposed to waterpipe smoking

Treatment with garlic extract reduced largely expression of p53 as demonstrated in figure (10).

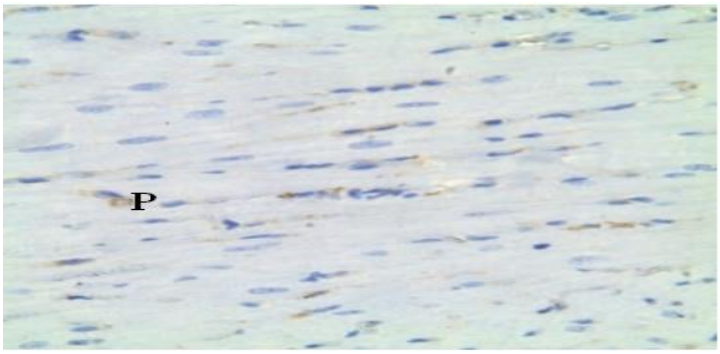

Figure 10: The effect of treatment with garlic extract on expression of p53 in the heart ventricle of waterpipe smoking exposed rat

DISCUSSION

This study was conducted considering increasing prevalence of smoking worldwide with its associated risks. Smoking has been reported to cause many diseases including cardiovascular, pulmonary diseases, and cancer (He et al., 2020; Mallah et al., 2023). There is a need to study the adverse effects associated with smoking on histological levels and to examine the effect of using some natural products rich in antioxidants such as garlic extract for ameliorating the side effects of smoking.

Smoking induced histological changes in the heart ventricular tissue. These changes included partial disruption of cardiac muscle fibers. Inflammatory changes were observed including lymphocytic infiltration. Exposure of rats to the smoking led to extensive changes in the heart ventricle. It is possible that these changes are due to chemical substances in smoking such as nicotine and carbon monoxide (CO) that have the potential impact on the function of cardiovascular system. These may include several mechanisms such as oxidative stress, endothelial alterations in structure and function, increased levels of cholesterol and triglycerides, and decreased levels of the high-density lipoprotein (HDL). Following intravascular inflammation by smoking, atherosclerosis and cardiovascular disease are likely to develop (Papathanasiou, et al., 2014).

However, nicotine has other impacts on the function of cardiovascular system including deregulation of cardiac autonomic function, boosting sympathetic activation, increasing heart rate, inducing coronary and peripheral vasoconstriction (Benowitz and Gourlay, 1997).

Smoking prompts the release of excessive amounts of free radicals that cannot be regulated and may cause dangerous mutations that destroy cells and induce oxidative stress. Smoking also impacts the pathway in which nitric oxide is reduced leading to endothelial dysfunction (Barua, et al., 2001).

Treatment with garlic extract in this study was protective against damaging effects of smoking.

We studied expression of p53 using immunohistochemical techniques in the heart. The results indicated to very low-level expression of p53 in the heart ventricle tissue. Both types of smoking models under study increased expression of p53 in the heart. The importance of our data comes from the roles that the p53 gene plays as a tumor suppressor gene which implies that its expression prevents the formation of tumors (Liu et al., .2022). Within the cell, p53 protein binds DNA, and acts to stimulate another gene ending with the production of a protein call smoke exposeded p21 which, in turn, inhibits further progression of real stage of cell divisions (Appella and Anderson, 2001; Aubrey et al., 2016).

In case there is a mutation in p53 gene, mutant p53 lacks the ability to bind DNA in an effective way, and the further steps to interfere with inhibiting cell divisions are not likely to occur effectively (Guo et al., .2024). According to this background of p53, the damaging effect of smoking either cigarette smoking or waterpipe smoking on the cellular environment is obvious. In general, treatment with garlic extract showed good expression against damaging molecular effects of mutated p53.

CONCLUSIONS

Cigarette and waterpipe smoking, is associated with adverse health effects. Smoking effects were studied on histological changes of the heart ventricle, using light microscope, and immunohistochemistry. Treatment with garlic extract showed ameliorate effects against adverse effects of smoking.

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