

## ORIGINAL ARTICLE

AMELIORATIVE EFFECTS OF MELATONIN ON  
HISTOPATHOLOGICAL CHANGES IN LUNG TISSUE OF  
NICOTINE-EXPOSED RATSShazia Parveen Channar, Sana Kashif\*, Sheeraz Ansari\*\*, Tariq Feroz Memon\*\*\*,  
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**Background:** Melatonin, a potent naturally occurring antioxidant plays a significant role in counteracting various toxins. This study aimed to evaluate the ameliorative effects of melatonin on histopathological changes in lung tissues of nicotine-exposed rats. **Methods:** This quasi-experimental study was conducted at Isra University, Hyderabad during Feb-May 2024. Forty Wistar albino rats were used. Rats were evenly divided into four groups. Group A (control group) received only normal chow, Group B received nicotine hydrogen bitartrate 0.6 mg/Kg bodyweight daily intra-peritoneal, Group C received same dose of nicotine with melatonin 5 mg/Kg I/P daily, and Group-D received the same dose of nicotine with melatonin 10 mg/Kg I/P. Serum inflammatory and antioxidant markers were analysed along with the histopathological analysis. **Results:** A significant rise in haematological parameters was observed in Group B compared to the control ( $p<0.05$ ). Group D showed more prominent decline in these parameters ( $p<0.05$ ) compared to Group C. A significant rise in c-reactive protein levels (from  $0.10\pm 0.11$  to  $0.88\pm 0.25$  mg/dL) and fibrinogen levels (from normal  $224.7\pm 74$  to  $447.1\pm 0.41$  mg/dL) were observed in Group B rats. The anti-oxidative and tissue peroxidative makers (MDA, SOD and GSH) levels were lower in Group C and D compared to control group, however, Group B showed a significant ( $p<0.05$ ) decrease in these. **Conclusion:** Melatonin has a protective effect against inflammatory, oxidative and proliferative reactions resulting from nicotine to lung tissues.

**Keywords:** Anti-inflammatory, Anti-oxidative, Lung tissue, Melatonin, Nicotine

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## INTRODUCTION

The lung is an organ composed of a complex network of tubes. It takes part in several vital physiological processes, such as gas exchange and immunological reactions. Respiratory infections are prevalent and represent a substantial danger to public health.<sup>1</sup> Over the past 10 years, there has been a rise in the number of lung disease-related deaths, particularly chronic obstructive pulmonary disease (COPD), which is brought on by the harmful effects of cigarette smoking.<sup>1</sup> Since cigarette smoke is directly inhaled into the lungs, it is one of the main risk factors for damage to multiple organs, particularly the lungs, which is the cause of death from cardiorespiratory failure.<sup>2</sup> Cigarette smoking causes one-third of all cancer-related deaths and ranks as a primary cause of lung cancer.<sup>3</sup> Nicotine is one of the over 9,500 components of cigarette smoke. The primary pharmacologically active ingredient in tobacco smoke's particle phase and the highly poisonous molecule that causes the majority of harmful effects of smoking, is nicotine.<sup>4</sup> By producing reactive oxygen free radicals, nicotine damages the lungs oxidatively. This frequently leads to a drop in the levels of glutathione peroxidase, catalase, and superoxide dismutase.<sup>5</sup>

The pineal gland produces the endogenous hormone melatonin (N-acetyl-5-methoxytryptamine)

primarily from the amino acid tryptophan. It shares structural similarities with serotonin and is secreted in the dark in both mammals and humans.<sup>6</sup> Its exogenous dietary sources include melatonin-rich foods like orange juice, walnuts, and sour cherries.<sup>7</sup> It has anti-inflammatory, anti-proliferative, anti-cancerous, anti-apoptotic (in injured cells), and pro-apoptotic (in malignant cells) qualities in addition to its potent antioxidant qualities. Even when administered before or concurrently with chemotherapy and radiation therapy, it has synergistic effects.<sup>8,9</sup>

Melatonin effectively suppresses inflammatory mediators, including IL-6, TNF- $\alpha$ , and MMP-9, as well as inflammatory cells, to achieve its anti-inflammatory effects.<sup>10</sup> Melatonin helps in preventing lung fibrosis brought on by cigarette smoke. This is achieved primarily by decreasing the production of TGF- $\beta$ 1 and collagen 1 and concurrently increasing the production of antioxidants glutathione peroxidase and superoxide dismutase.<sup>11</sup>

Being a naturally occurring hormone, melatonin is far safer to use both therapeutically and prophylactically than other medications. It is also widely accessible and has very few adverse effects. Research on humans and animals has demonstrated its safety for short-term and long-term treatments.<sup>6</sup>

The primary objective of the present study was to evaluate the ameliorative effects of melatonin on histopathological changes in the lung tissue of nicotine-exposed rats.

## MATERIAL AND METHODS

After obtaining approval from the Ethical Committee, this quasi-experimental animal study was conducted jointly by the Departments of Biochemistry and Pharmacology, Isra University, Hyderabad, from 5 Feb to 30 May 2024. Animal procedures were performed at Sindh Agriculture University, Tandojam, Hyderabad. Forty rats were selected through the non-probability consecutive sampling technique. The sample size was determined using power analysis.<sup>12</sup>

The procured Wistar albino rats were housed in separate cages with free access to food and water. The temperature and light/dark cycles were maintained at standard levels.<sup>13</sup> The animals were divided randomly into 4 groups (A, B, C, and D), each comprising of 10 rats. For 4 weeks, Group A (Control group) was given regular saline. Group B (nicotine-exposed group) rats received daily nicotine injections at 0.5 mg/Kg through intraperitoneal administration while the rats experienced cigarette smoke exposure for 30 minutes every day inside a glass chamber. Group C received melatonin via oral administration at a dose of 5 mg/Kg daily, while the rats received nicotine at 0.5 mg/Kg through intraperitoneal injection and experienced cigarette smoke for 30 minutes each day. Group D received melatonin through oral administration at 10 mg/Kg daily dose, while the rats received nicotine at 0.5 mg/Kg through intraperitoneal injection and experienced cigarette smoke for 30 minutes each day.

At the end of the 4<sup>th</sup> week, all rats were kept fasting for 18 hours and sacrificed by decapitation. Fasting blood samples of 2 ml in EDTA-tube were collected. The blood samples from each rat were collected from the retrograde orbital plexus before the sacrifice in a capillary tube under anaesthesia. The collected blood samples were sent to the Isra University Diagnostic Laboratory for estimation of Red blood cells (RBCs), Haemoglobin (Hb), White blood cells (WBCs), and Platelets, along with C-reactive protein (CRP), Fibrinogen, Interleukin-6 (IL-6) and Tumour Necrosis Factor alpha (TNF- $\alpha$ ). After employing commercially available kits obtained from BioAssay Technologies China, the analyses of Superoxide Dismutase (SOD) activity, Malondialdehyde (MDA), and Glutathione GSH levels were carried out.

Lung specimens of the rats for histological investigation were taken after dissection and preserved in 10% formalin. The slides for histological investigation were processed and stained.<sup>14</sup> The

distinctive histological alterations and findings of lung samples were recorded on a pre-designed proforma. Data were entered and analysed on SPSS-25. The Mean $\pm$ SD of various parameters for each group were noted. ANOVA followed by post-hoc Tukey was applied, and  $p\leq 0.05$  was considered statistically significant.

## RESULTS

Table-1 presents the post-hoc Tukey test of haematological parameters in groups. Rats in Group B had significantly higher RBC counts, Hb concentrations, WBC count, and Platelets at the end of the experiment, compared to control group ( $p<0.05$ ). Group D rats showed a significant reduction in haematological parameters including RBCs, Hb concentration, WBC, and platelet count, compared to Group C.

In comparison to Group B, melatonin administration effectively decreased the serological inflammatory markers in Group C and D ( $p<0.05$ ). The levels of CRP, fibrinogen, IL-6 and TNF- $\alpha$  levels decreased in both treatment groups. However, these reductions were more pronounced in Group D than in Group C for all markers ( $p<0.05$ ) (Table-2).

Table-3 shows the oxidative stress marker levels (MDA, SOD, GSH) across all study groups. Group B showed significantly elevated MDA while depleting SOD and GSH compared to Group A ( $p<0.05$ ). A moderate-dose melatonin (Group C) provided intermediate MDA restoration, whereas high-dose (Group D) nearly normalized it. Moreover, the nicotine-induced reduction in SOD and GSH activity was significantly mitigated by the administration of melatonin in different doses.

Figure-1 depicts the histo-microphotographs of lung tissue sections of all study groups. Histopathological analysis of the lungs of Group A rats showed regular histological architecture for the bronchial tree and alveoli. However, the lung tissue of the animals in Group B showed notable histological changes, such as inter-alveolar septal hypertrophy (blue arrow) and proliferation, as well as mononuclear cell infiltration and fibrous growth. There was perivascular oedema, hyalinization patches (orange star), and a small amount of inflammatory cell infiltration in the blood vessels (yellow arrow). Group B rats also exhibited thickening of the interalveolar septa, bronchial epithelial necrosis, and desquamation. Group C showed good responses to all histopathology variables of detrimental effects of nicotine exposure such as increased cellularity and partially preserved alveolar spaces (green arrows), to the low dose of melatonin treatment. A more prominent damage protecting effect to the nicotine was observed after the administration of a 10 mg/Kg dose of melatonin in the treatment Group D (yellow arrow).

**Table-1: Haematological parameters across study groups (Mean±SD)**

Test Parameters	Groups				p
	A	B	C	D	
RBCs (million/mm <sup>3</sup> )	4.47±0.29 <sup>B</sup>	5.28±0.22 <sup>A</sup>	4.93±0.20	4.72±0.43 <sup>B</sup>	0.004
Hb (g/dL)	8.28±0.21 <sup>B,C,D</sup>	10.26±0.32 <sup>A,D</sup>	9.87±0.03 <sup>A,D</sup>	9.01±0.65 <sup>A,B,C</sup>	0.000
WBCs (Thousand/mm <sup>3</sup> )	24.21±1.15 <sup>B,D</sup>	30.89±1.81 <sup>A,C,D</sup>	28.63±1.21 <sup>A,B</sup>	27.32±1.88 <sup>B</sup>	0.000
Platelets (10 <sup>4</sup> /mm <sup>3</sup> )	35.42±1.89 <sup>B,C</sup>	41.85±1.43 <sup>D</sup>	39.21±2.10 <sup>A</sup>	37.87±1.12 <sup>B</sup>	0.000

A, B, C, and D indicate significant differences in groups (ANOVA followed by post-hoc Tukey,  $p \leq 0.05$ )

**Table-2: Serum Inflammatory markers levels in all groups (Mean±SD)**

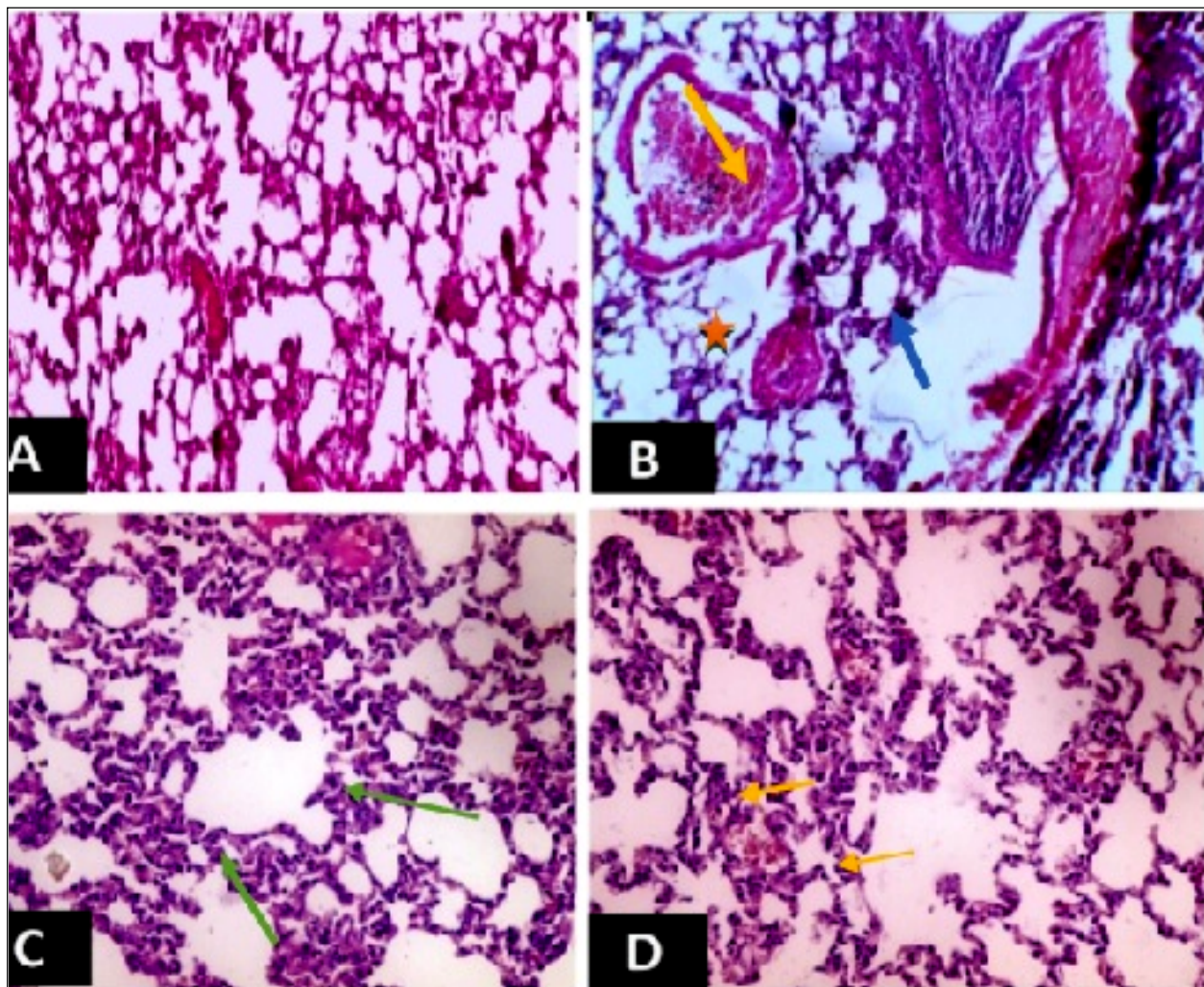
Test Parameters	Groups				p
	A	B	C	D	
Serum CRP (mg/dl)	0.11±0.09 <sup>B</sup>	0.85±0.19 <sup>A,D</sup>	0.61±0.21	0.29±0.38 <sup>B</sup>	0.002
Serum Fibrinogen (mg/dl)	231.5± 68 <sup>B,C</sup>	450.3±0.37 <sup>A,C,D</sup>	292.3±0.19 <sup>A,B</sup>	321.2±0.51 <sup>B</sup>	0.000
IL-6 (pg/ml)	108.33±5.1 <sup>B,C,D</sup>	172.16±3.0 <sup>A,C,D</sup>	168.41±2.5 <sup>A,B,D</sup>	151.45±5.4 <sup>A,B,C</sup>	0.000
TNF- $\alpha$ (pg/ml)	105.2±5.1 <sup>B,C,D</sup>	276.4±6.3 <sup>A,B,D</sup>	221.8±5.5 <sup>A,B,D</sup>	158.6±4.8 <sup>A,B,C</sup>	0.000

A, B, C, and D indicate significant differences in groups (ANOVA followed by post-hoc Tukey,  $p \leq 0.05$ )

**Table-3: Oxidative stress markers level across all groups (n=40)**

Test Parameters	Groups				p
	A	B	C	D	
MDA (nmol/g tissue)	127.55±0.45 <sup>D</sup>	159.13±1.01	143.45±0.87 <sup>D</sup>	130.21±1.22 <sup>A,C</sup>	0.000
SOD (U/mL)	1129.36±100.23 <sup>C,D</sup>	213.71±0.21 <sup>C,D</sup>	714.19±49.27 <sup>A,D</sup>	937.31±28.74 <sup>A,C</sup>	0.000
GSH (mg/L tissue)	4.87±0.84 <sup>B</sup>	2.43±1.02 <sup>A,D</sup>	4.13±1.34	4.48±1.11	0.006

A, B, C, and D indicate significant differences in groups (ANOVA followed by post-hoc Tukey,  $p \leq 0.05$ )



**Figure-1: Histopathological microphotograph of lung tissue sections of all groups**

## DISCUSSION

Tobacco smoking is widespread worldwide. Nicotine, an ingredient in cigarettes, is harmful to human health and often causes an increase in mortality. Pineal gland's hormone melatonin is essential for preserving the health of both people and animals.

Our study demonstrated that there was a significant rise in RBCs, WBCs, Haemoglobin, and Platelets in the nicotine-induced Group B compared to the control group. This is due to the toxic effects of cigarette smoke on blood flow and vessel health as well as on blood elements such as WBCs and platelets.<sup>15</sup> Melatonin-injected Groups C and D showed significant protection from the abnormally alterations of haematological parameters. This normalization is prominent in Group D compared to Group C. These findings are consistent with Wang *et al*<sup>16</sup>, Ngai *et al*<sup>17</sup>, Kulsoom *et al*<sup>18</sup>, and Zhao *et al*<sup>19</sup>, all of whom studied the potential protective effects of melatonin on lung tissues.

Our study observed elevated levels of CRP, Fibrinogen, IL-6 and TNF- $\alpha$  in the rats induced with nicotine only compared to all other groups. These findings are consistent with those reported by Siddiqui *et al*<sup>20</sup>, Centner *et al*<sup>21</sup>, and Khaled *et al*<sup>22</sup>. According to the American Heart Association (AHA), cigarette smoking is linked with the expression of pro-inflammatory cytokines, particularly the activation of toll-like receptor 4 (TLR4)-inflammasome-IL-6 signalling axis, which in turn leads to an inflammatory cascade and ultimate release of different inflammatory cells and proteins including CRP and fibrinogen, etc.<sup>23</sup> The co-administration of melatonin in different doses decreased the levels of these inflammatory markers. This reduction was more pronounced in group D, though these levels did not return to control levels.

The ameliorative effects of melatonin against this raised level of inflammatory markers are reported by different studies.<sup>23-25</sup> In addition to the anti-inflammatory properties, melatonin exhibits antioxidant potential against various toxic chemicals, especially nicotine. In the present study, we demonstrated that nicotine caused a significant decline in anti-oxidative markers in lung tissue. Countering this effect, treatment with 5 mg/Kg melatonin somewhat alleviated the nicotine-induced drop in MDA, SOD, and GSH activity. However, the decline was offset by co-administration of 10 mg/Kg of melatonin. Andersson *et al*<sup>24</sup>, Lim *et al*<sup>25</sup>, and Wang *et al*<sup>26</sup>, reported the potential role of melatonin against the oxidative stress induced by nicotine in lung tissue.

The present study emphasizes how melatonin may have protective benefits in reducing the pulmonary tissue injuries caused by nicotine. Notwithstanding, it is imperative to recognize certain

constraints, such as the absence of evaluation of lipid profile, Cotinine levels, bronchoalveolar lavage and gene expression, which may have yielded more insights regarding the plausible anti-inflammatory and antioxidant properties of melatonin.

## CONCLUSION

Higher doses (10 mg/Kg) of Melatonin have an effective property against inflammatory, oxidative and proliferative reactions resulting from nicotine in lung tissues. In cases of persistent nicotine-induced lung tissue damage, melatonin might be helpful. Further studies are recommended, including more parameters like lipid profile, and even gene studies, which will provide more in-depth findings.

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### Contribution of Authors:

Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

**SPC:** Concept, design, drafting, final approval

**SA:** Concept, design, data collection and assembly, drafting of article

**QAS:** Data collection and assembly, drafting of article

**SK:** Concept, design, data collection and assembly, drafting of article

**TFM:** Analysis and interpretation of the data and drafting of article

**HA:** Data collection and assembly, drafting of article

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