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Research Article
Protective Effects of Spirulina Platensis- Selenium Nanoparticles Against Nicotine Induced-Lung Toxicity

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Abstract

Background and Objective: Smoking contributes significantly to the development of cardiovascular disease and lung cancer in smokers. The purpose of this study was to look into the protective effect of Spirulina platensis nanoparticles (SP-SeNPs) on nicotine-induced lung toxicity in mice. **Materials and Methods:** SP-SeNPs were prepared and characterized to find mean particle size of SP-SeNPs and zeta potential. Furthermore, the IC_{50} of SP-SeNPs against the A549 lung carcinoma cell line and LD_{50} was calculated. A total of 90 adult albino mice weighing approximately 35±5 g was used in this study. **Results:** Results showed that the shapes of SP-SeNPs were spherical and the mean particle size was 39.86±0.14 nm and negative zeta potential was +33.14. Also, IC_{50} of SP-SeNPs against the A549 lung carcinoma cell line and LD_{50} was 72.7 μg mL^{-1} and 490 mg kg^{-1} body weight, respectively. The daily oral administration of SP-SeNPs at concentrations of 9.8 and 24.5 mg kg^{-1} body weight for 30 days to mice treated with nicotine (2.5 mg kg^{-1} body weight) resulted in a significant improvement in plasma total cholesterol (TC), triacylglycerols (TG), high density lipoprotein-cholesterol (HDL-C), phospholipids (PLs) and vitamin C, as well as lung TNF-α, interleukin 6 (IL-6), interleukin 10 (IL-10). On the other hand, inducible oral administration of SPSNPs, increased the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx), decreased glutathione (GSH) and the level of malondialdehyde (MDA) in nicotine-treated lungs of mice. Furthermore, SP-SeNPs almost normalized these effects in lung histoarchitecture. **Conclusion:** The SP-SeNPs have lung protective activity against nicotine-induced lung toxicity in mice.

Key words: Spirulina platensis, SP-SeNPs, nicotine, lung toxicity, cytokine storm

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nicotine is classified as an alkaloid and meets the criteria for a highly addictive drug[1]. Nicotine is not only a stimulant but also a depressant[2,3]. Nicotine also increases total cholesterol (TC), triacylglycerols (TG) and low-density lipoprotein-cholesterol (LDL-C) serum levels[4,5]. The excessive production of reactive oxygen species (ROS) may lead to oxidative stress[6]. These ROS in turn, promote lipid peroxidation in oxidative damage[7]. Cotinine (main metabolite of nicotine) is produced by liver C-oxidation pathway and used as a nicotine-intake marker[8]. Kidney is probably the organ which removes cotinine from the liver. Many plants are believed to have highly antioxidant effects[9], an important characteristic of medicines associated with the treatment of various diseases[10].

Spirulina is classified as a "Superfood". It is a truly amazing food, full of nutritional wonders[11]. Spirulina platensis and Spirulina maxima are the two most common Spirulina species used in nutritional supplements[12]. Spirulina, contains large amounts of protein and carotenoids, omega 3 or omega 6 polyunsaturated fatty acid, Gamma linolenic acid, sulpholipids, glycolipids, potassium, zinc and selenium, vitamin A, vitamin E and a variety of minerals[13]. Nanoparticles can reduce side effects in patients by targeting the area of disease directly and removing the need of circulation throughout the body[14,15]. Researchers have observed improved drug solubility, controlled release, increased organic bioavailability, increased stability and better long-term storage when encapsulating drugs into nanoparticles (versus non-encapsulated drugs)[16]. These attributes are promising and necessary for fighting against diseases[17]. Medicinal significance of natural products was evaluated in some previous studies[9,17,18], the present study was conducted to provide an easy route for assessing the therapeutic potential of lung toxicity by nicotine for SP-SeNPs in mice.

MATERIALS AND METHODS

Materials: Spirulina platensis water extract Powder (100%) was purchased from Zazzee naturals, USA. All other chemicals used in this study were of the analytical grade. Fine chemicals [Selenious acid (H_2SeO_3) (Aldrich), ascorbic acid (99%, Aldrich)] were of analytical grade.

Synthesis of Spirulina platensis in-situ Selenium Nanoparticles (SPSNPs): A 20 mM ascorbic acid (Vc) solution was freshly prepared by dissolving 35.2 mg Vc powder in 10 mL of Milli-Q water. Spirulina platensis water extract (SPWE) was dissolved in deionized water and diluted in deionized water (90 mL) in a conical flask as follows: Dissolved selenious acid (H_2SeO_3, 0.013 g, 0.01 mmol.) in 10 mL deionized water was added to the solution, with continuous stirring and heating at 60°C for 10 h; forming in-situ after which 200 L of 40 mM ascorbic acid was added as a catalyst; the ruby red SeNPs were suspended and characterized by transmission electron microscopy (TEM).

Spirulina platensis in-situ Selenium Nanoparticles (SPSNPs) characterization: The crystal-line characteristics and grain dimensions of SP-SeNPs were determined by the X-ray diffraction pattern at 25-28°C with nickel (Ni) (D8 Advance X-ray diffractometer) filtered using CuKα (β = 1.54184 A0) radiation as X-rayed source. Scanning electron microscope and field transmission microscope at an accelerating voltage of 15 and 200 Kv have investigated the morphology and size of the SP-SeNPs.

Determination of SP-SeNPs cytotoxicity on cells: The 96 well tissue culture plate was inoculated with 1×10^5 cells mL^{-1} (100 uL $well^{-1}$) and incubated at 37°C for 24 h to form a complete monolayer sheet. After forming a confluent sheet of cells, growth medium was decanted from 96 well micro titer plates and the cell monolayer was washed twice with wash media. Two-fold dilutions of the tested sample were made in RPMI medium with 2% serum (maintenance medium). In each well, 0.1 mL of each dilution was tested, with three wells serving as controls and receiving only maintenance medium. The plate was incubated at 37°C and then examined.

Cells were checked for any physical signs of toxicity, e.g. partial or complete loss of the monolayer, rounding, shrinkage, or cell granulation. MTT solution was prepared (5 mg mL^{-1} in PBS) (BIO BASIC CANADA INC). 20ul MTT solution were added to each well. Place on a shaking table, 150 rpm for 5 min, to thoroughly mix the MTT into the media. Incubate (37°C, 5% CO_2) for 1-5 h to allow the MTT to be metabolized. Dump off the media (dry plate on paper towels to remove residue if necessary). Resuspend formazan (MTT metabolic product) in 200 uL DMSO. Place on a shaking table, 150 rpm for 5 min, to thoroughly mix the formazan into the solvent. Read optical density at 560 nm and subtract background at 620 nm. Optical density should be directly correlated with cell quantity.

Animals: A total of 90 male albino mice weighing approximately 35±5 g (90 mice, 60 for LD_{50} estimation and 30 mice for estimation of SPNPs lung protective activity) were

Table 1: Description of treatment groups

Groups	Group names	Treatments description
1	Normal control A	3 mL of distilled water, orally for 30 days
2	Nicotine	Subcutaneous injection of 2.5 mg kg^{-1} body weight nicotine[20] in water, for 30 days
3	SP-SeNPs+Nicotine	Oral suspension of 1/50 LD50 (9.8 mg kg^{-1} body weight SPSNPs) in water+subcutaneous injection of 2.5 mg kg^{-1} body weight nicotine in water for 30 days in a single daily dose
4	SP-SeNPs+Nicotine	Oral suspension of 1/20 LD50 (24.5 mg kg^{-1} body weight SPSNPs) in water+subcutaneous injection of 2.5 mg kg^{-1} body weight nicotine in water for 30 days in a single daily dose
5	5FU+Nicotine	Intraperitoneal injection of 20 mg kg^{-1} body weight 5FU by on alternate days for 3 weeks[21]+subcutaneous injection of 2.5 mg kg^{-1} body weight nicotine in water for 30 days in a single daily dose

obtained from the animal house of Cairo University, Giza, Egypt. They were housed in plastic cages with stainless steel covers at the National Cancer Institute Animal House. In a light-controlled room, the animals were kept at a temperature of 21±2°C and a humidity of 55-60%. The animals were kept for one week to acclimate and were given *ad libitum* access to feed and water.

Determination of LD_{50} of SPSNPs: Preliminary tests were performed on groups of four mice. SP-SeNPs were administered orally in various doses to determine the range of doses that cause zero to 100% mortality in animals. The LD_{50} was determined in groups of ten animals by orally administering resveratrol nanoemulsion at different doses of 100, 200, 400, 600, 800 and 1000 mg kg^{-1}. Animals were observed individually every hour for the first day and every day for the next five days following administration of the tested SPSNPs. Throughout the experiment, animals' behaviour and clinical symptoms were recorded. Method of Finney[19] was used to calculate the LD_{50} using the following formula:

$$LD_{50} = DM - \left[\frac{\sum(Z \times d)}{n}\right]$$

Dm : The largest that kill all animals
$\sum$: The sum of (z×d)
Z : Mean of dead animals between 2 successive groups
d : The constant factor between 2 successive doses
n : Number of animals in each group

Experimental setup: This experiment was carried out to examine the protective effect of SP-SeNPs against nicotine-induced lung toxicity. This experiment was conducted in accordance with guidelines established by the Animal Care and Use Committee of October 6 University. The Research Ethics Committee at the Faculty of Applied Medical Sciences, October 6 University in Egypt, granted ethical approval for data collection (No. 20201202). There were no human subjects used in the studies that served as the foundation for this research; instead, mice were used in an *in vivo* study. Adult albino mice were divided into six groups with six animals in each. The treatment groups are described in Table 1.

After 30 days of treatment, blood samples were drawn from retro-orbital vein and collected in heparin-containing tubes. The heparinized blood samples were centrifuged for 20 min at 1000 xg. The separated plasma was used to calculate plasma cholesterol[22], triglycerides[23], cholesterol-high density lipoprotein (HDL)[24], phospholipids[25] and vitamin C[26].

II-Preparation of lung samples: Cervical dislocation was used to kill the animals and then the lungs were quickly removed. To prepare a 25% W/V homogenate, a portion of each lung was weighed and homogenised in a glass homogenizer (Universal Lab. Aid MPW-309, mechanika precyzyjna, Poland) with ice-cold saline. Three aliquots of the homogenate were prepared. The first was deproteinized with ice-cold 12% trichloroacetic acid and the supernatant obtained after centrifugation at 1000 xg was used to calculate decreased glutathione (GSH)[27].

The second aliquot was centrifuged at 1000 xg and the supernatant was used to calculate the levels of malondialdehyde (MDA)[28], nitric oxide (iNOs)[29], tumour necrosis factor-alpha (TNF-α)[30], interleukin-6 (IL-6) and interleukin-10 (IL-10)[31]. The third aliquot of homogenate was used to prepare a cytosolic fraction of the lung by centrifuging it at 10500 xg for 15 min at 4°C in a cooling ultra-centrifuge (Sorvall comiplus T-880, Du Pont, USA) and the clear supernatant (cytosolic fraction) was used to determine the activities of superoxide dismutase (SOD)[32] and glutathione peroxidase (GPx)[33].

Histological assessment: The lung was cut into pieces and fixed in a 10% buffered formaldehyde solution for histological study. An automated tissue processing machine was used to process the fixed tissues. Tissues were embedded in paraffin wax using standard techniques. Sections of 5 lm thickness were prepared and stained with hematoxylin and eosin for

light microscopy analysis using the Bancroft and Steven method[34]. Following that, the sections were examined under the microscope for histopathological changes and photomicrographs were taken.

Statistical analysis: Data were analyzed using one-way analysis of variance (ANOVA), followed by the least significant difference test using the Statistical Package for Social Science (SPSS) version 18.0 for windows (SPSS, Inc., IBM, Chicago, Illinois, USA)[35]. The results were expressed as Mean±SD. Values of $p<0.05$ were considered statistically significant.

RESULTS

Transmission electron microscopy (TEM) analysis showed that the size of SP-SeNPs was around 39.86±0.14 nm with negative zeta potential of +33.14 (Fig. 1).

Figure 2 showed that the IC_{50} of SP-SeNPs against A549 lung carcinoma cell line = 72.7 µg mL^{-1}.

Table 2 shows that oral administration of SP-SeNPs at the doses of 100, 200, 400, 600, 800 and 1000 mg kg^{-1} body weight resulted in mortalities of 0, 2, 4, 7, 9 and 10 respectively. The dose of SP-SeNPs that killed half of the mice (LD_{50}) was 490 mg kg^{-1} body weight.

Table 3 shows plasma TC, TG, HDL-C, phospholipids (PLs) and vitamin C levels. Intraperitoneally administration of nicotine led to significant increase in TC and TG while significantly decreased the HDL-C, PLs and Vit. C, respectively, as compared to the normal control group ($p<0.05$), indicating acute lung injury. Treatment of animals with SP-SeNPs at 9.8 and 24.5 mg kg^{-1} body weight, as well as 5FU (20 mg kg^{-1} body weight) significantly decreased the level of TC and TG as well as significantly increased HDL-C, PLs and vitamin C, respectively, ($p<0.05$), as compared to the nicotine treated group. The effect of SP-SeNPs at two different doses was more pronounced than 5FU.

Table 4 shows a significant increase in lung TNF-α, iNOs and IL-6 as well as significant decrease in IL-10 levels ($p<0.05$) in mice treated with nicotine compared to the control group. The administration of SP-SeNPs at 9.8 and 24.5 mg kg^{-1} body weight as well as 5FU 20 mg kg^{-1} body weight showed significant decrease in TNF-α, iNOs and IL-6 as well as significant increase in IL-10 levels compared with the nicotine treated group of mice after 30 days ($p<0.05$).

Table 5 shows a significantly ($p<0.05$) decreased activities of lung antioxidant parameters (SOD, GPx and GSH) while significant increase in lung MDA was observed in the nicotine-treated mice as compared to the normal control group ($p<0.05$), indicating acute lung damage. The administration of SP-SeNPs at 9.8 and 24.5 mg kg^{-1} body weight significantly ($p<0.05$) increased the lung enzymes activities (SOD, GPx and GSH) in mice and decreased MDA level, as compared to the nicotine-treated group. Also, 5FU treatment has not significantly changed when compared to nicotine treated group.

Histopathological examination of lung sections of the normal group 1 showed normal morphological features of lung parenchyma with apparent intact respiratory airways epithelium as well as alveolar walls (arrows) with intact vasculatures (Fig. 3).

On the other hand, in the lung of nicotine-treated control group (2), histological examination showed complete lung collapse; note the brochostenosis (arrows), interstitial blood vessel congestion (*) and mononuclear cells infiltration (arrowhead), (H&E X400). Histopathological examination also showed good recovery of nicotine-induced lung toxicity (II) by

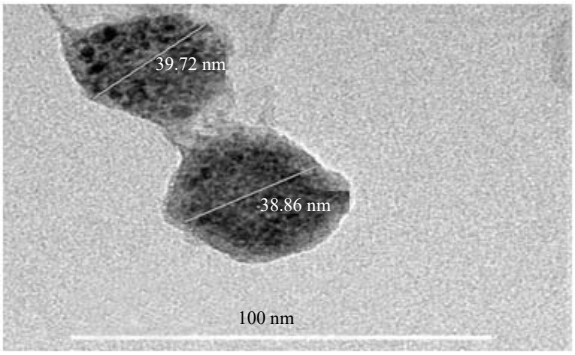

Fig. 1: Transmission electron microscopy (TEM) analysis of SP-SeNPs

Table 2: Determination of LD_{50} of resveratrol nanoemulsion given orally in adult mice

Group no.	Dose (mg kg^{-1})	No. of animals/group	No. of dead animals	Z	d	Z×d
1	100	10	0	1.0	100	100
2	200	10	2	3.0	200	600
3	400	10	4	5.5	200	1100
4	600	10	7	8.0	200	1600
5	800	10	9	8.5	200	1700
6	1000	10	10	0.0	00	5100

$LD_{50} = DM - \left[\frac{\Sigma(Z \times d)}{n}\right]$, $LD_{50} = 1000 - \left[\frac{5100}{10}\right] = 490\,mg\,kg^{-1}$ body weight

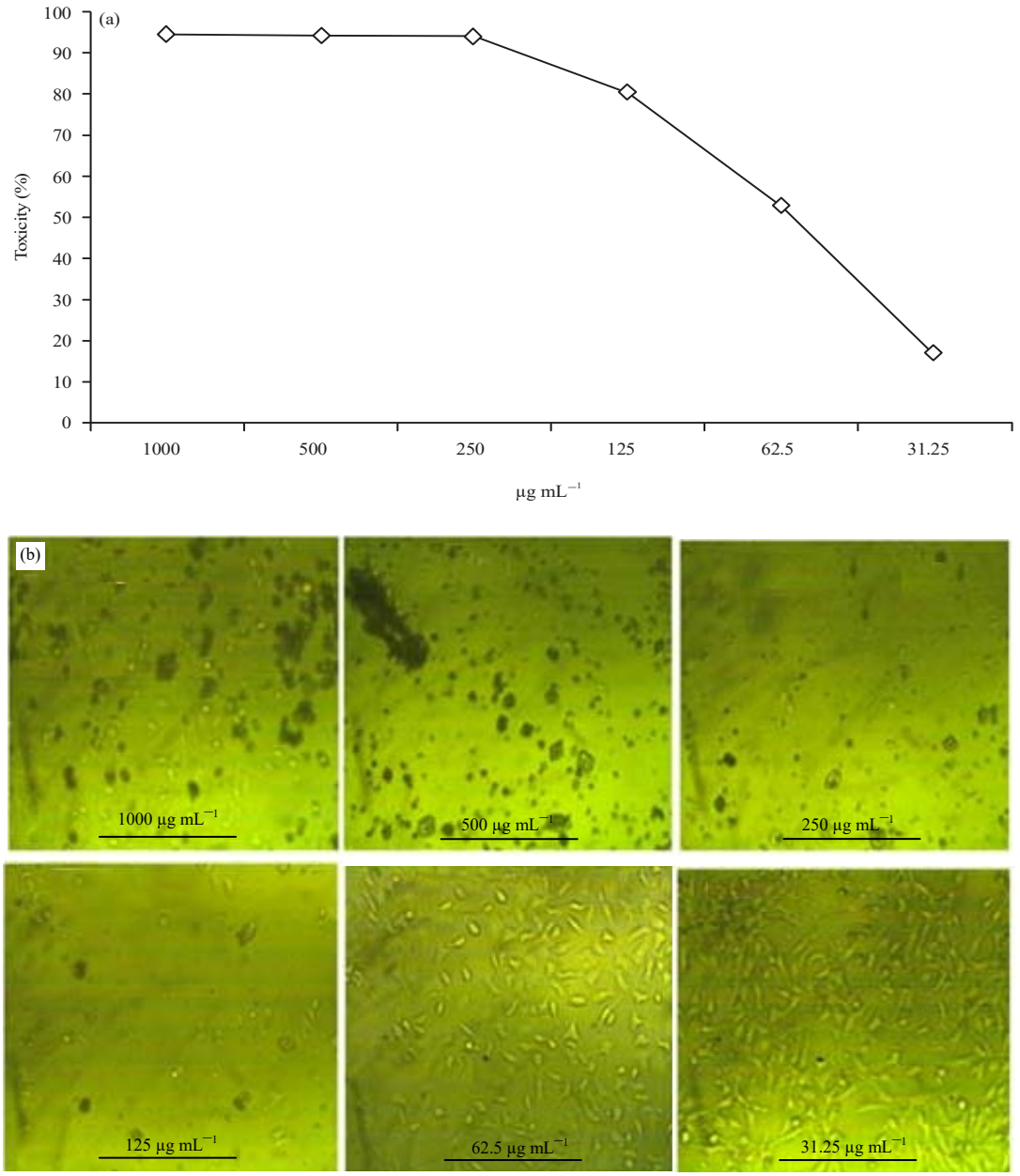

Fig. 2(a-b): IC_{50} and the effect SP-SeNPs against A549 lung carcinoma cell line

Table 3: Effect of SP-SeNPs on plasma TC, TG, HDL-C, PLs and vitamin C in mice treated with nicotine

Groups	Treatments description	TC (mg dL^{-1})	TG (mg dL^{-1})	HDL-Cl (mmol L^{-1})	Pls (mg dL^{-1})	Vitamin C (mg dL^{-1})
1	Normal control A	235.80±11.76^{a}	133.70±14.51^{a}	42.89±3.90^{c}	63.87±5.38^{c}	95.48±3.46^{c}
2	Nicotine (2.5 mg kg^{-1} b.w.)	293.76±7.60^{d}	186.09±13.25^{d}	29.06±3.76^{a}	42.59±3.81^{a}	64.38±5.96^{a}
3	SP-SeNPs (9.8 mg kg^{-1} b.w.)+Nicotine (2.5 mg kg^{-1} b.w.)	251.47±12.65^{c}	140.79±9.69^{b}	35.98±4.21^{b}	57.66±.94^{c}	83.27±6.28^{b}
4	SP-SeNPs (24.5 mg kg^{-1} b.w.)+Nicotine (2.5 mg kg^{-1} b.w.)	238.70±8.77^{a}	135.42±19.25^{a}	39.39±3.27^{c}	61.80±5.11^{c}	97.98±5.08^{c}
5	5FU(20 mg kg^{-1} b.w.)+Nicotine (2.5 mg kg^{-1} b.w.)	267.56±15.40^{b}	149.80±10.54^{c}	33.87±4.22^{b}	49.43±2.60^{b}	65.25±6.48^{a}

Results are presented as Mean± standard deviation of number of observations within each treatment. Mean values followed by same letters are not significantly different at $p \leq 0.05$., b.w.: Body weight

SP-SeNPs at 9.8 and 24.5 mg kg^{-1} body weight as compared to the nicotine-treated group and showed almost the same records as groups 3 and 4.

In group V, all samples of nicotine treated mice recovered by treatment with 5FU and showed improvement in the lung parenchyma; note the absence of collapse with

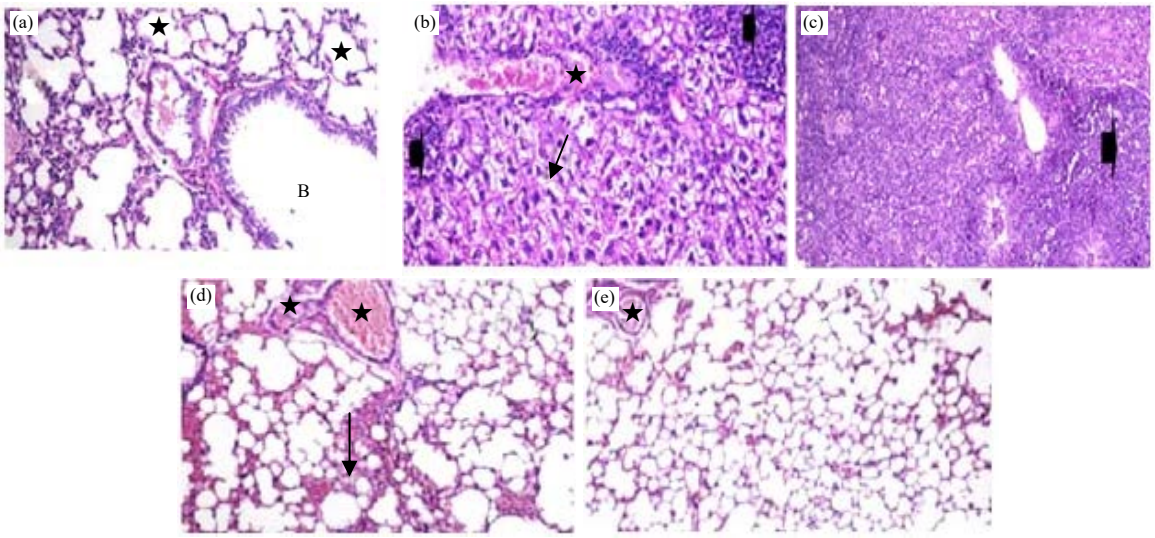

Fig. 3(a-e): Sections stained with hematoxylin and eosin (H&E; 400×) histological examination of mice' lungs of different groups compared to control group (a) Group 1: Normal control, (b) Group 2: Nicotine (2.5 mg kg^{-1} body weight), (c) Group 3: SP-SeNPs (9.8 mg kg^{-1} b.w.) + Nicotine (2.5 mg kg^{-1} b.w.) was administrated, (d) Group 4: SP-SeNPs (24.5 mg kg^{-1} b.w.) + Nicotine (2.5 mg kg^{-1} b.w.) was administrated, (e) Group 5: 5FU (20 mg kg^{-1} b.w.) + Nicotine (2.5 mg kg^{-1} b.w.) was administrated

Table 4: Effect of SP-SeNPs on levels of lung TNF-α, iNOs, IL-10 and IL-6 in mice treated with nicotine

Groups	Treatment description	TNF-α (pg mL^{-1})	iNOs (pg mL^{-1})	IL-6 (pg mL^{-1})	IL-10 (pg mL^{-1})
1	Normal control A	9.65±0.93^{a}	21.09±2.76^{a}	43.87±3.11^{a}	18.76±2.08^{c}
2	Nicotine (2.5 mg kg^{-1} b.w.)	25.43±3.26^{a}	36.20±3.08^{a}	89.53±5.22^{a}	9.44±0.85^{a}
3	SP-SeNPs (9.8 mg kg^{-1} b.w.)+Nicotine (2.5 mg kg^{-1} b.w.)	14.50±1.27^{b}	28.74±3.62^{a}	64.07±4.81^{a}	13.26±0.98^{b}
4	SP-SeNPs (24.5 mg kg^{-1} b.w.)+Nicotine (2.5 mg kg^{-1} b.w.)	12.80±1.60^{d}	24.00±2.89^{c}	49.65±3.47^{d}	15.89±2.70^{c}
5	5FU (20 mg kg^{-1} b.w.)+Nicotine (2.5 mg kg^{-1} b.w.)	18.77±1.64^{b}	29.80±2.44^{b}	55.43±4.97^{b}	12.00±1.16^{b}

Results are presented as Mean±standard deviation of number of observations within each treatment. Mean values followed by same letters are not significantly different at p≤0.05., b.w.: Body weight

Table 5: Effect of SP-SeNPs on levels of lung superoxide dismutase (SOD) and glutathione peroxidase (GPx), malondialdehyde (MDA) and reduced glutathione (GSH) in mice treated with nicotine

Groups	Treatment description	SOD	GPx	MDA (nmol mg^{-1} protein)	GSH (mg%)
1	Normal control A	19.54±3.20^{d}	17.65±0.84^{c}	0.98±0.12^{a}	12.25±0.84^{c}
2	Nicotine (2.5 mg kg^{-1} b.w.)	8.70±0.87^{a}	4.77±0.79^{a}	2.15±0.27^{d}	5.48±0.32^{a}
3	SP-SeNPs (9.8 mg kg^{-1} b.w.)+Nicotine (2.5 mg kg^{-1} b.w.)	13.46±0.65^{c}	8.70±0.44^{b}	1.43±0.22^{c}	10.18±0.55^{c}
4	SP-SeNPs (24.5 mg kg^{-1} b.w.)+Nicotine (2.5 mg kg^{-1} b.w.)	18.00±1.65^{d}	15.87±0.38^{c}	1.09±0.18^{a}	11.35±1.51^{c}
5	5FU(20 mg kg^{-1} b.w.)+Nicotine (2.5 mg kg^{-1} b.w.)	10.34±2.54^{a}	4.66±0.66^{a}	1.90±0.70^{a}	6.73±0.84^{a}

Results are presented as Mean±SD for groups of six animals each. Mean values followed by same letters are not significantly different at p≤0.05. SOD: One unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in 1 min mg^{-1} protein, GPx: µg of GSH consumed min^{-1} mg protein, b.w.: Body weight

moderate congestion in the peri-bronchial blood vessels (*) and peri-alveolar blood capillaries (arrows), (H&EX100).

DISCUSSION

Tobacco use is one of the leading causes of cardiovascular disease, including coronary heart disease, ischemic stroke, peripheral artery disease and abdominal aortic aneurysm[36]. It is also linked to an increased risk of certain cancers and is a leading cause of chronic obstructive pulmonary disease[37].

In the current study, nicotine-treated mice had significantly higher levels of TC and TG during the experimental period than those of the normal control mice. Nicotine, on the other hand, lowers plasma levels of HDL-C, PLs and vitamin C, which is a powerful protective factor against the development of atherosclerosis[37].

Furthermore, oral administration of SP-SeNPs at 9.8 and 24.5 mg kg^{-1} body weight, as well as 5FU at 20 mg kg^{-1} body weight, provided significant protection against nicotine-induced increases in plasma TC and TG.

The deposited cholesterol esters in the tissue need hydrolysis to release free cholesterol. One of the hydrolysis factors is HDL-C, since HDL-C level was found to be decreased in mice fed with atherogenic diet[38], the insufficient HDL-C level may lead to free cholesterol in plasma, enhancing the pathogenesis. Also, certain natural products can enhance lipid profiles in mice treated with toxic compound[39]. Hussein *et al.*[40], also, reported that spirulina species can reduced the LDL in nicotine treated rats. LDL promotes atherosclerosis both by providing lipids signals that initially activate macrophages and by stimulating foam cell formation[40]. These effects could be due to presence of carotenoids, omega 3 or omega 6 polyunsaturated fatty acid, Gamma linolenic acid, sulpholipids, glycolipids, potassium, zinc and selenium, vitamin A, E and a variety of minerals in SPSNPs.

The current study showed that SP-SeNPs could normalise lung levels of TNF-, IL-6, IL-10 and iNOS in the nicotine-treated group. Nicotine-induced free radicals regulate cell proliferation and death, as well as gene expression of TNF-, IL-6, iNOS and MDA[9].

Evidence suggests that free radicals, oxidative stress and lipid peroxidation are present in organ damage[41]. It has been demonstrated that in chronic lung toxicity, increased lung concentrations of TNF-, IL-6, iNOS and MDA, as well as decreased activity of IL-10, SOD, GPx and GSH, induced mitochondrial toxicity and free radical generation[42].

The most extensively studied mitogenic and fibrogenic factors are TNF-, IL-6, IL-10 and iNOS. SP-SeNPs can also inhibit the expression of proinflammatory cytokines[43]. These findings suggest that SPSNPs' antifibrotic effect is linked to the inhibition of mitogenic and/or fibrogenic signalling. TNF- has been shown to stimulate the formation of NO[44].

SP-SeNPs are a powerful reactive oxygen species (ROS) scavenger[45] and normalised the oxidative stress biomarkers SOD, GPx, GSH and MDA, resulting in decreased oxidative stress, which contributes to nicotine's suppression of lung inflammation. In the current study it was observed that after nicotine administration, there was a significant decrease in lung SOD, GPx and GSH activity.

Endothelial activation was also elicited by IL-10 and iNOS protein expression, which could be mitigated by spirulina extract[40]. SP-SeNPs inhibited H_2O_2-induced monocyte adhesion to HCAECs in a similar concentration range, which is significant. The second significant finding was that resveratrol inhibits TNF-induced NF-B activation in HCAECs[46]. Other studies, suggested that spirulina extract was effective against iNOS protein expression, IL-10 and TGF-1-induced NF-B activation in intact blood vessels[40].

According to histological studies, SP-SeNPs have a lung-protective effect. Because lung proliferation is an early event in toxicity-related changes, the attenuation of lung injury and fibrosis in mice by SP-SeNPs could be associated with a reduction in inflammatory response. To the best of my knowledge, the prophylactic effect of SP-SeNPs against nicotine-induced lung toxicity has never been reported and this study may be the first of its kind.

CONCLUSION

The current study found that SP-SeNPs have potent lung protective activity against nicotine-induced lung toxicity by normalizing the levels of oxidative stress biomarkers and inflammatory mediator gene expression.

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