Ki-67 pulmonary immunoreactivity in silver nanoparticles toxicity: Size-rate dependent genotoxic impact

Sanaa A. Ali a, Mai O. Kadry a,⁎, Olfat Hammam b, Sohair A. Hassan a, Rehab M. Abdel-Megeed a

a Therapeutic Chemistry Department, Pharmaceutical and Drug Industries Research Institute, National Research Center, El Buhoush St., Dokki, Cairo 12622, Egypt
b Pathology Department, Theodor Bilharz Research Institute, Egypt

Abstract

Engineered nanoparticles have been recently utilized in numerous domains particularly, silver nanoparticles (AgNPs). Nonetheless, the possible side effects resulting from AgNPs exposure are not fully clarified. The present study was designed to clarify the toxicity of AgNPs on lung tissue. Furthermore, therapeutic impact of Glycosmis pentaphylla (G. pentaphylla) and Casimiroa edulis (C. edulis) leaves extracts in addition to mucilage and protein (the purified compounds from C. edulis) was investigated against AgNPs induced pulmonary toxicity. Male Swiss albino mice were administered AgNPs orally in two different particle sizes (20 nm and 100 nm) for one month and was further treated via G. pentaphylla, C. edulis, mucilage and protein in a dose of 500 mg/ kg for three weeks. Biochemical, molecular, immunohistochemistry, and histopathological investigations were further assessed. An obvious alteration in oxidative stress biomarkers as well as mRNA gene expression of both survivin and matrix metalloproteinase (MMP-9) was recorded in AgNPs intoxicated group. In addition to, exploration of positive nuclei for Ki-67 was also observed upon AgNPs intoxication. Data declared a significant improvement in the assessed parameters upon G. pentaphylla, C. edulis, mucilage and protein treatment. In conclusion; G. pentaphylla and C. edulis extracts could be considered as a promising candidate as therapeutic regime against pulmonary toxicity induced via AgNPs due to their enrichment with different active constituents.

Practical applications: Due to the expansion of AgNPs applications, it is urgent to investigate their toxic impact associated with release of free silver ions. Different particle sizes of AgNPs can induce various alterations in cellular biochemical parameters, mRNA gene expression, histopathological and immunohistopathological examination. Herein, this natural products extracts are used for the first time as promising therapeutic regimen to ameliorate the toxic effect in AgNPs intoxicated lung tissue in mice model as a result of the bioactive metabolites, especially flavonoids and polyphenolic compounds.

1. Introduction

Silver nanoparticles (AgNPs) are commonly utilized in several consumer products. Due to their high antimicrobial property, AgNPs are recommended for attenuating bacterial as well as fungal contamination of food packaging, textiles, implants, cosmetics, and medical devices [55,73]. Despite the wide range of AgNPs industrial applications, it recorded numerous inflammatory and organs dysfunctions properties [63]. AgNPs can exist as an airborne dust therefore, exposure levels should be put in consideration regarding AgNPs size that was inhaled [19,58]. Numerous studies investigated high inflammatory potential associated with AgNPs accumulation in the lung and may lead to granulomatous lesions. Furthermore, AgNPs from the lung can permeate epithelial barrier via the blood and/or the lymphatic fluid by its bio-kinetic property into remote tissues such as kidney, liver, and spleen, causing various toxic effects [29,65,75]. AgNPs impact was previously assessed in vitro [23,43,71]. Numerous cell lines recorded apoptosis or necrotic cell death within AgNPs acute exposure as a result of reactive oxygen species accumulation, leading to lipid peroxidation, oxidative generated DNA lesions, mutations of genes, mitochondrial swelling, leakage of lysosome, and finally blockage of autophagic flux [33,35,51,70,71] On the other hand, chronic exposure to AgNPs triggers inflammatory and thiol responses, causing damage in mitochondria, phagocytic capacity reduction, and increment in nitric oxide levels through lipopolysaccharide stimulation [13].

Survivin, is an inhibitor member of apoptosis family through...
inhibition of caspase-mediated cell death by reduction of caspase gene expression in which, it binds to the apoptotic X-linked inhibitor and the pro-apoptotic “Smac/DIABLO” factor that leads to releasing Smac/DIABLO in cytoplasm [15,62]. Furthermore, survivin plays an essential role in cell cycle during the G2/M phase [21,39]. Many human tumors express high levels of survivin, and its tissue level is closely related to tumor progression and poor prognosis [52]. However, over expression of survivin in embryonic stage is downregulated in adult tissues, some healthy adult cells declared expressed survivin [17]. Therefore, survivin overexpression is essential during regeneration of tissue and its suppression may be due to injury [66].

Matrix metalloproteinases (MMPs) are zinc-established enzymes which are responsible for degradation of extracellular matrix proteins, even though they are responsible for activation or suppression numerous molecules [28]. Un-regulation of the MMP gene expression is associated with pathogenesis of various lung diseases [16,20,64].

Immunohistochemical analysis is not widely applied for the detection of lung toxicity. However, Ki-67 immunostaining assay has been investigated to detect cellular dysfunction [44,57,9]. The Ki-67 antigen exists in the nucleus but in incomplete form. It was reported to be expressed in all phases of the cell cycle except G0 [57]. Epithelial cells that can express Ki-67 are mainly elevated in bronchial cells indicating that it may be an important indicator in lung inflammation, apoptosis and lung cancer [32]. As previously reported, level of ki-67 notably increased in both current and ex-smokers [74]. Furthermore, Ki-67 was investigated as an early diagnostic biomarker for lung cancer [72].

Herein, we supposed that the Ki-67 immunohistochemistry index could be overexpressed in pulmonary epithelial cells upon AgNPs intoxication based on different sizes in mice model. In addition, detection of both survivin and MMP-9 as molecular biomarkers is related to lung dysfunction. Casimiroa edulis (C. edulis) tree, which is known as “white sapota” is cultivated in Egypt. C. edulis leaves and seeds are previously being eaten for their calming activities and helping to sleep [56]. Moreover, the leaves extract was investigated as hypnotic, anticonvulsant, diuretic, antihypertension, antioxidant, anti-inflammatory, anti-Alzheimer and anti-carcinogenic agent [41,45,5,8]. Furthermore, different compounds were extracted from leaves and seeds of C. edulis, G. pentaphylla extracts, protein and mucilage of C. edulis seeds and fruits respectively [5,34].

2. Materials and methods

2.1. Chemicals and reagents

AgNPs of two particle sizes (20 nm and 100 nm) were purchased from Sigma-Aldrich Co. (St Louis, Missouri, USA). Kits used for biochemical analyses were obtained from Bio-diagnostic (Biotic diagnostic Co., Egypt). Kits for total RNA extraction, primers and RT-PCR SYBR green kits were obtained from Qiagen (Germany-Helden). All other biochemical analyses were obtained from Biodiagnostic (Biodiagnostic Co., Egypt). Kits for total RNA extraction, primers and RT-PCR SYBR green kits were obtained from Qiagen (Germany-Helden). All other chemicals were of high analytical grade.

Distribution of AgNPs size was analyzed with a Brookhaven 90 Plus particle size analyzer. Transmission electron microscopy (TEM) was subjected for the evaluation of AgNPs size.

2.2. Plant material and extract preparation

Leaves of C. edulis and G. pentaphylla were collected from a botanical Al-Orman garden, Giza, Egypt and Mohammed Ali museum, respectively. Extraction of both C. edulis and G. pentaphylla was previously established in addition to, the active constituents, protein and mucilage of C. edulis seeds and fruits respectively [5,34].

2.3. Experimental design

2.3.1. Animal groupings and treatments

One hundred-ten male Swiss Albino mice (20–25 g /each) were obtained from the National Research Center (NRC), Cairo, Egypt. Animals were kept in cages for acclimatization and allowed free access to feed and water ad libitum. All animals received adequate care and handling according to institutional animal ethics committee of NRC (18193).

After acclimatization (one week), animals were randomly subdivided into eleven groups (10 /each) as follows:

Group 1: Healthy animals.

Group 2: untreated mice [25] that were orally given AgNPs in low size (20 nm) for one month and served untreated as positive control for 20 nm AgNPs [25].

Groups 3, 4, 5 & 6: AgNPs 20 nm intoxicated animals which were treated with C. edulis, G. pentaphylla extracts, protein and mucilage of C. edulis seeds and fruits respectively at a dose of 500 mg/kg /each for one month [5].

Group 7: untreated mice that were orally given AgNPs in high size 100 nm [25] for one month and served untreated as positive control for 100 nm AgNPs.

Groups 8, 9, 10 & 11: AgNPs 100 nm intoxicated animals which treated with C. edulis, G. pentaphylla, extracts protein and mucilage of C. edulis respectively at a dose of 500 mg/kg /each for one month [5].

2.4. Determination of some antioxidants

Activity of antioxidant was determined in serum samples for all tested groups. SOD (Superoxide Dismutase) and GST (Glutathione S-transferase) were measured. Buller et al., ($year$) [14,50].

2.5. RNA extraction and RT-PCR analysis for apoptosis determination

Lung samples of each group were used to extract total RNA individually according to the manufacturer’s instructions illustrated in Qiamp mini kit (Qiagen; USA; Cat No. 74104). Complementary DNA (cDNA) and mRNA gene expression of Survivin and MMP-9 forward and reverse primer sequence; 5'-GTGCTGGGCTGCTGCTTTGCTG-3' and 5'-GTGCCGGTCAAAGGTTTGGAAT-3' quantitative RT-PCR were assessed using RT-PCR master mix SYBR green kit (Qiagen; USA; Cat No. 204243). Followed by real time PCR, reaction amplification was carried out in Stratagene Mx3000 P QPCR System (Agilent Technologies, Santa Clara, CA, USA) in 20 μl reaction volume. Primers sequences are listed in Table 1. Temperature profile was calculated according to optimum annealing temperature for each primer. The relative expression of survivin and MMP genes was compared by comparative CT (2^-ΔΔCt) method.

| Table 1 | Primers sequence designed for RT-PCR gene expression. |
|---------|--------------------------------------------------------|
| Primer name | Primer sequence |
| B- actin | 5-CTTGATGTGACGGGACGATTC-3 |
| | 5-GGCGCGTCAAGGACCAA-3 |
| MMP-9 | 5'-GCCACATCTGGCCCTTGAGTC-3' |
| | 5'-CTTCCAGAGATGGCCACTGCT-3' |
| Survivin | 5'-GGGAATTTGGAACACTGGACAG-3' |
| | 5'-CCCTTCTAAGATGGTCTAAG-3' |
against the expression of β-actin as reference gene [2].

2.6. Immunohistochemistry Determination of Ki-67

Lung tissue Section (4 μm thick) were prepared for Ki-67 antigens immunohistochemistry (IHC) detection. Tissue sections were kept in 0.03 % hydrogen peroxide (H₂O₂) at room temperature for 10 min, due to the removing of endogenous peroxidase activity. After that, serum [(0.5 % normal goat serum X0907, Dako Corporation, Carpinteria, CA, USA,) and (0.04 % bovine serum albumin, A2153, Sigma-Aldrich, Shanghai, China)] was added to block the reaction at room temperature for 30 min. Ki-67 Anti-body (Roche, CONFIRM anti-Ki-67 (30–9) Rabbit Monoclonal Primary Antibody: 790–4286) was added (at a dilution of 1:200) then incubated overnight at 4 °C. After that, sections were subjected to washing three times for 5 min in PBS. Non-specific staining then was blocked using 5 % normal serum at room temperature for 30 min. Finally, staining was evaluated with diaminobenzidine as a substrate and sections were counterstained with hematoxylin. PBS replaced the antibody in negative controls [3].

2.7. Histopathology

Lung tissues were preserved and fixed in 10% of formalin buffer to form representative slices. After that, samples were embedded in paraffin 4-μm thick slides were then stained by hematoxylin and eosin (H&E). Finally, slides were examined under a light microscope [31].

2.8. Statistical analysis

All the obtained data were analyzed using SPSS version 16 software (USA). All values were expressed as the mean ± SE. Significance of differences among all groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparisons post hoc test where p < 0.05 was considered significant.

3. Results

3.1. Characterization studies

AgNPs had a mean hydrodynamic diameter potential of 20.58 ± 1.5 nm and 100.62 ± 0.9 nm. The size of the two sized nanoparticle demonstrated by TEM was 20 ± 2 nm and 100 ± 12 nm. Fig. 1 represented TEM image of AgNPs of both 20 nm and 100 nm respectively.

3.2. Oxidative stress biomarkers estimation

AgNPs (20 nm and 100 nm) intoxicated animal investigated a significant reduction in SOD values as well as GST levels as compared to negative healthy animals (Fig. 1) with observation that declared that 20 nm AgNPs induced more oxidative stress than those of 100 nm. Treatment with C. edulis indicated a significant elevation in SOD of both 20 nm as well as 100 nm AgNPs groups. However, treatment of 100 nm AgNPs -intoxicated groups with G. pentaphylla, extracts, protein and mucilage of C. edulis declared non-significant improvement of oxidative stress biomarker of SOD and GST values (Figs. 2 and 3).

3.3. Modulation of survivin mRNA gene expression

AgNPs in 20 nm particle sized intoxicated mice demonstrated a significant elevation in survivin gene expression (3.1 fold change) compared to negative healthy controls (Fig. 3). Meanwhile, AgNPs 100 nm in particle size did not declare an obvious change and there weren’t any recorded significance values. Treatment with C. edulis indicated a significant downregulation in survivin gene expression with a 0.8 fold change. However, treatment of 20 nm AgNPs intoxicated groups with G. pentaphylla, declared non-significant improvement of survivin gene expression (2.8 fold change). Furthermore, mucilage declared a significant downregulation of survivin gene expression (1.1 fold change) as compared to negative control. Meanwhile, protein extract investigated a significant downregulation of survivin gene expression (1.5 fold change) (Fig. 4).

3.4. Regulation of MMP-9 mRNA gene expression

Twenty nm AgNPs (20 nm) particle sized intoxicated group recorded a significant elevation in MMP-9 gene expression (3.7 fold change) as compared to healthy mice group as well as a significant elevation was declared upon 100 nm AgNPs intoxication (1.87 fold change). Treatment with C. edulis indicated a significant downregulation in MMP-9 gene expression (1.51 fold change). Furthermore, treatment of 20 nm AgNPs intoxicated groups with G. pentaphylla, showed a significant improvement of MMP-9 gene expression recording a 1.61 fold change. However, treatment 20 nm AgNPs by protein recorded non-significant improvement (3.1 fold change). On the other hand, a significant improvement was declared upon protein treatment followed by 100 nm AgNPs (1.5 fold change) (Fig. 5).
3.5. Ki-67 immunohistochemistry detection

Immunohistochemistry determination for Ki-67 of lung section from negative control mice declared negatively stained Ki-67. However, section of samples obtained from AgNPs (100 nm) illustrated nearly 10% of pneumocytes and investigated positive nuclei for Ki-67. Moreover, AgNPs (20 nm) intoxicated group demonstrated 40% of pneumocytes showed positive nuclei for Ki-67 (Fig. 6; part I).

Treatment of AgNPs (20 nm) with C. edulis indicated nearly 10% of pneumocytes showing positive nuclei for Ki-67. Whereas, lung section of AgNPs (20 nm) treated with G. pentaphylla showed about 7% of pneumocytes showing positive nuclei for Ki-67. Lung section of AgNPs (20 nm) treated mucilage investigated about 2% of pneumocytes showing positive nuclei for Ki-67. Furthermore, lung section from AgNPs (20 nm) treated protein group did not have any positive staining of Ki-67 (Fig. 6; part II).

Lung section of AgNPs (100 nm) intoxicated group followed by C. edulis treatment showed negative staining of Ki-67. Section from
AgNPs (100 nm) lung tissue treated with glycosmis, declared about 15% of pneumocytes showed positive nuclei for Ki-67. Section from AgNPs (100 nm) treated lung by mucilage, showed negative staining of Ki-67. Section of lung tissue for AgNPs (100 nm) treated protein showed negative staining of Ki-67 (Fig. 6; part III).

3.6. Histopathological investigation

Histopathological examination of lung section from negative control group demonstrated normal alveoli with thin inter-alveolar septum, type I and type II pneumocytes were also detected, in addition to blood vessel and bronchus as shown in section A, lung section illustrated positive control AgNPs (20 nm) showing alveoli with mild thick inter-alveolar septum which was infiltrated by a mild number of lymphocytes and RBCs. Furthermore, type I and type II pneumocytes were also seen and ruptured alveoli as showed in section B. On the other hand, lung section from intoxicated AgNPs (100 nm) group, showing alveoli with markedly thick inter-alveolar septum, the septum was infiltrated by a large number of lymphocytes and RBCs ruptured alveoli as demonstrated in section C (Fig. 7; part I).

Upon C. edulis treatment of AgNPs (20 nm), lung section showed alveoli with moderate thick inter-alveolar septum, the septum was infiltrated by a moderate number of lymphocytes and RBCs ruptured alveoli and bronchus were declared as illustrated in section A. Meanwhile lung section of AgNPs (20 nm) treated with G. pentaphylla, showed almost normal alveoli with thin inter-alveolar septum, type I and type II pneumocytes were also seen in section B: lung section of AgNPs (20 nm) treated mucilage, showing near normal alveoli with thin inter-alveolar septum, type I and type II pneumocytes were also seen, blood vessel, bronchus as demonstrated in section C. Moreover, lung section from AgNPs (20 nm) treated protein, declared alveoli with mild thick inter-alveolar septum.
Furthermore, the septum is infiltrated by mild number of lymphocytes and RBCs, type I and type II pneumocytes were also seen in addition to ruptured alveoli and bronchus as representative in section D (Fig. 7; part III).

As demonstrated in section A, lung samples of AgNPs (100 nm) by treated C. edulis, observed almost normal alveoli with thin inter-alveolar septum, type I and type II pneumocytes were also observed. Lung section from intoxicated AgNPs (100 nm) followed by treatment with G. pentaphylla, showed alveoli with mild-moderate thick inter-alveolar septum, the septum is infiltrated by mild number of lymphocytes and RBCs. Furthermore, type I and type II pneumocytes and ruptured alveoli were also observed (B). On the other hand, lung section from AgNPs (100 nm) treated mucilage, showed alveoli with mild thick inter-alveolar septum, the septum is infiltrated by mild number of lymphocytes and RBCs, type I and type II pneumocytes and ruptured alveoli were also observed (C). Meanwhile, lung section of protein treated group showed almost normal alveoli with thin inter-alveolar septum, type I and type II pneumocytes were also observed, blood vessel and bronchus as shown in section D (Fig. 7; part III).

4. Discussion

Prevalence of AgNPs application in medicine as well as industry increased the risk of their accumulation in tissues and organs causing toxic effects on internal organs [6]. Most studies on lung toxicity are conducted by inhalation, but during our oral route experiment, it was found that mice had difficulty in breathing with enlarged lung size, so the present study evaluated the effect of AgNPs toxicity on lung tissue via monitoring biochemical and histological variations. In general, the lung is of particular concern, in that it is the most vulnerable organ to nanoparticles intoxication post exposure via oral treatment or inhalation.

In the current study, AgNPs intoxication in mice model of both low and high sizes (20 nm and 100 nm) declared lung inflammation as well as genotoxic effects due to accumulation of AgNPs in the lung tissue confirming toxicity. Furthermore, AgNPs (20 nm) demonstrated more toxic effect than AgNPs (100 nm). Moreover, immunohistochemistry and histopathological alternation was monitored in lung tissue, and oxidative stress besides survivin and MMPs mRNA overexpression.

AgNPs toxic effect was determined according to its physical as well as chemical properties according its size [22]. In previous study, AgNPs ranging from 10 to 20 nm cause a considerable toxic effect [40]. As Ag⁺ ions are related to the cell death, it is urgent to consider the number of Ag⁺ ions in the inventor suspension of AgNPs [12,54]. Furthermore, it has been verified that endocytosed AgNPs can be degraded in lysosomes releasing Ag⁺ ions in the cytosol and cause further cell death.

The oxidative stress has been enhanced by injection of AgNPs (20 nm and 100 nm) in the present study. AgNPs contamination contributed to oxidative stress that resulted in a significant reduction in SOD as well as GST. Previous studies also observed the increment of oxidative stress in aquatic creatures in heavy metal contaminated aquatic environment [37]. However, AgNPs supplementation with limited dose (0.5 mg/kg) could improve the obtained oxidative stress; high dose of AgNPs (1 mg/kg) increase the oxidative stress validating that AgNPs induce toxicity by the effect of Ag⁺ ions [7].

Herein, the current study deduced the up regulation of survivin mRNA in AgNPs induced lung injury in experimental animals. It was also shown that upon treatment with AgNPs 20 nm treated groups via C. edulis and mucilage improved mRNA gene expression of survivin overexpression. Detection of survivin indicates that it could be
considered as an important mediator of cyto-protection, not only in tumor cells but also in lung injured adult cells. Previously, it was confirmed that survivin gene expression was altered in injured lung epithelial cells in both *vitro* and *vivo* via activation of caspase-3 and caspase-7 declaring the anti-apoptotic potential of survivin [27]. However, reports observed that survivin could regulate apoptosis and proliferation of both normal cells and cancer cells through similar pathways such as p21-dependent pathways [26]. Continued investigations regarding mechanisms that regulate the expression of survivin and its function in normal and tumor tissues can develop a novel therapeutic strategy [67].

Dysregulation of MMP-9 has been associated with acute lung injury [68]. In the current study, the expression profiles of MMP-9 gene in AgNPs (20 nm) intoxicated group of lung tissues were analyzed and declared overexpression of MMP-9 mRNA levels. On the other hand, AgNPs (100 nm) intoxicated group didn’t declare a significant change in MMP-9 expression confirming size dependent genotoxic impact of AgNPs. Our results demonstrated that the expression of MMP-9 was significantly overexpressed in AgNPs (20 nm) intoxicated group as indicated by increased pulmonary inflammation and oxidative stress. This finding suggested that pulmonary overexpression of MMP-9 may be a part of a self-protective response as previously reported by [42,76].

Ki-67, as a DNA-binding nuclear non-histone protein index is an appealing biomarker of lung cancer, where it was previously reported to be increased in lung cancer patients. Furthermore, it was also previously related to prognosis [49]. However, various investigations recorded an intensive variability in Ki-67 index among diverse individuals that reflect a difference in biological response to other injurious agents [60]. In addition, whereas the increment of Ki-67 index is associated with increasing pre-neoplasia histology, there is an extensive variation in intensive variability in Ki-67 index among diverse individuals that related to prognosis [49]. However, various investigations recorded an.

In the present study, sections obtained from AgNPs (100 nm) intoxicated group illustrated nearly 10% of pneumocytes and revealed positive nuclei for Ki-67. Whereas AgNPs (20 nm) intoxicated group demonstrated 40 % of pneumocytes showed positive nuclei for ki-67.
These results proved the AgNPs (20 nm) have a more toxic effect than that induced via AgNPs (100 nm).

Furthermore, the correlation between the expressions of Ki-67 in all studied groups was assessed. The results declared that the expression of Ki-67 is related to the powerful of all used regimen in treatment of injured lung samples. Data recorded indicated an obvious improvement in Ki-67 expression in both AgNPs (20 nm) and AgNPs (100 nm) with the superiority of mucilage-treated group confirming that Ki-67 is an attractive biomarker for lung injury related to AgNPs intoxication.

Treatment of AgNPs intoxicated groups with C. edulis, G. pentaphylla extracts. In addition to mucilage and protein bioactive constituents demonstrated a remarkable improvement in all tested biochemical parameters, apoptotic biomarker, and immunohistochemistry for Ki-67 in addition to histopathological examination. The noticeable elaboration is due to the antioxidative properties of these compounds as it increases their health benefits [11]. Herein, this beneficial impact may be due to the existence of active phenolic compounds such as phenolic acids, flavonoids, terpenoids and phenolic [34]. In the present study, G. pentaphylla extracts of stems and leaves showed obvious antimicrobial as well as antioxidant activities due to the presence of many valuable compounds such as phenolics, saponins, alkaloids and tannins. Furthermore, the antioxidant activity of these extracts is associated with a high content of flavonoids [46]. Different previous studies were concerned with studying the hepatoprotective effect of vigor of C. edulis and G. pentaphylla [34,38,4,53]. Here, we have shed light on the prospective role of the bioactive constituents present in the current extracts against lung toxicity induced via AgNPs. Rosmarinic acid and Gallic acid are phenolic compounds which can protect lung tissues against oxidative stress and pathological alternation for its antioxidant property, its functional property as scavenging reactive oxygen species and their power to improve body antioxidant status [5]. Different investigations identified more bioactive compounds with antioxidant properties in C. edulis due to the presence of phenolic compounds [24].

Additionally, the active constituent rutin could influence the inhibition of some cancers due to its antioxidant, anti-angiogenic, anti-allergic, anti-inflammatory, and antiviral properties [61]. Naringenin, a dietary flavonoid, improved the physiological alterations caused by isoniazid, certain metabolic alteration that declared anti-inflammatory and anti-oxidative properties [1].

In our study, many histopathological changes in the lung tissue of AgNPs intoxicated animal demonstrated alveoli with markedly thick inter-alveolar septum, the septum is infiltrated a large number of lymphocytes and RBCs ruptured alveoli declining necrosis, and cellular inflammation. These findings are previously recorded by Hassan and Abdelbaky, who reported damage and necrosis in the lung tissue upon AgNPs intoxication [30]. This damage and inflammation is due to the accumulation of silver ions in the lung tissue. Furthermore, treatment with C. edulis and G. pentaphylla showed an obvious improvement in lung tissue declaring thin inter-alveolar septum, type I and type II pneumocytes, blood vessel and bronchus. This improvement is due to the considerable antioxidative impact due to the presence of phenolic compounds such as flavonoids in addition to, cytotoxic and antimicrobial activities [34].

5. Conclusions

In summary, the current study proved the adverse action of exposure to AgNPs on lung tissue. Lung toxicity was induced via different pathways including oxidative stress, antioxidant mechanisms, induction of inflammation, and alteration of mRNA gene expression, in addition to overexpression of Ki-67. Furthermore, AgNPs intoxication caused alterations in lung histology. Treatment of intoxicated animals with G. pentaphylla, C. edulis extracts, mucilage and protein the active constituents investigated their antioxidative cytotoxic and antimicrobial activity due to their enrichment with phenolic compounds such as flavonoids.

In conclusion, these extracts and the two active constituents could be promising candidates as therapeutic regimen against lung toxicity induced by AgNPs. Moreover, these plants as functional foods, possess potential positive effects on health beyond main nutrition.

Ethics Approval

All experimental procedures were approved by the Animal Care and Ethical Committee of NRC. All authors have revised the manuscript and agree for its publication in toxicology reports journal.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Consent to Participate

Not applicable.

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