Ameliorative effect of polydatin and polydatin-loaded chitosan nanoparticles against diabetes-induced pulmonary disorders in rats

Fatma Mostafa a, Sanaa R. Galaly a, Hanaa M. Mohamed b, Adel Abdel-Moneim c and Manal Abdul-Hamid

a Faculty of Science, Histology and Cytology Division, Zoology Department, Beni-Suef University, Beni-Suef, Egypt; b Faculty of Science, Genetic and Molecular Genetic Division, Zoology Department, Beni-Suef University, Beni-Suef, Egypt; c Faculty of Science, Molecular Physiology Division, Zoology Department, Beni-Suef University, Beni-Suef, Egypt

ABSTRACT

The current study aims to evaluate the ameliorative effect of polydatin-loaded-chitosan nanoparticles (PD-CSNPs), polydatin (PD), and metformin on Diabetes-induced pulmonary disorders. After induction of diabetes, rats are classified into five groups, diabetic control and diabetic rats treated daily for four weeks with; PD, PD-CSNPs, chitosan-nanoparticles and metformin. Rats treated with PD and PD-CSNPs showed a reduction in glycosylated-haemoglobin % and lipid peroxidation level, increased activities of superoxide peroxidase, superoxide dismutase and catalase, and glutathione content in treated diabetic rats lung. Furthermore, PD-CSNPs, PD and MET decreased tumour necrosis factor-alpha and nuclear factor-kappa-β and upregulated nuclear factor erythroid 2-related factor-2 and haem oxygenase-1. Histological and ultrastructural examinations revealed their ameliorative effect via reducing the bronchiolar and alveolar fibrosis, thickening of the interalveolar septum, and inflammatory cell infiltration. In conclusion, PD-CSNPs was more effective than MET and PD in improvement the diabetes-induced pulmonary disorders through its antioxidant, anti-inflammatory, and prolonged-release properties.

1. Introduction

Diabetes mellitus (DM) develops when the individual had insulin resistance or insulin release diminished [1]; induces hyperglycaemia that produces reactive oxygen species (ROS) and disables the defenses of antioxidant cells [2]. DM may cause adverse complications in the respiratory system, termed as DM-induced pulmonary disorders [3] due to systemic inflammation, oxidative stress, hypoxaemia, and direct harm caused by chronic hyperglycaemia [4]. Additionally, the key preventive factor against DM-induced pulmonary complications is a large microvascular reserve. Micro- and macrovascular dysfunctions due to diabetes develop later in the lungs than in other organs because of such large pulmonary reserve. [5]. ROS and advanced end products for glycation initiate proinflammatory response and endothelial dysfunction through activating nuclear factor-kappa-β (NF-κβ) [6]. Nuclear factor erythroid 2-related factor 2 (Nrf2) performs a pivotal protective function in the lung, where stimulation of Nrf2 maintains the intracellular redox balance and avoids oxidative cell injury [7]. Nrf2 deficiency causes lung damage-associated with mitochondrial biogenesis and mitophagy deficiencies in alveolar type 2 cells, also increased gene expression-encoding the inflammatory cytokine tumour necrosis factor [8]. DM induces irregular lung histological changes, as cellular infiltration, inflammation, and collagen accumulation [9]. Also, diabetes causes abnormal ultrastructural changes as capillary endothelial cells with numerous plasmalemmal vesicles on a thickened blood-air barrier [10]. The diabetic complications that are caused by hyperglycaemia seem to be due to an imbalance between ROS, leading to oxidative stress and cellular death [11]. Antioxidants have been shown to scavenge free radicals and ROS [12]. Polydatin (PD) is a major active component of Polygonum cuspidatum Sieb. and Zucc. (Polygonaceae) that is a traditional Chinese medicine and glycoside of resveratrol [13]. Previous studies have shown that polydatin exerts several pharmacological effects, including anti-inflammation [14] and anti-oxidant activities [15]. The PD, therefore, modulate lipid and glucose metabolism to alleviate diabetes [16–18] as well as has antioxidant potential which can help to protect the body against oxidation and several diabetes complications [19]. PD-loaded chitosan nanoparticles (PD-CSNPs) illustrated a marked antidiabetic efficacy than free PD in diabetic rats [20]. Metformin is derived from galegine, a natural product from the plant Galega.
officinalis, used in herbal medicine in medieval Europe. Galegine was tested as a glucose-lowering agent in humans in 1920s but was found to be too toxic [21]. Various studies have revealed the role of metformin in antioxidant processes via induced significant elevation in oxidized glutathione along with a reduction in glutathione/oxidized glutathione ratio [22]; so metformin is usually used in T2DM as antidiabetic therapy and also is important in reducing oxidative stress [23]. DM-related complications in multiple organs were addressed markedly, but the mechanisms related to DM-induced pulmonary disorders had been limited. Therefore, the study aims to assess the possible ameliorative mechanisms of PD-CSNPs against DM-induced pulmonary disorders compared to PD and metformin through biochemical, histopathological, immunohistochemical, and ultrastructural investigations.

2. Materials and methods

2.1. Animals and materials

Forty-eight male Wistar albino rats of body weight between 100 and 140 g, were obtained from the Egyptian Organization for Biological Vaccine Production breeding unit (VACSERA, Cairo, Egypt). Rats were kept in well-aerated cages at suitable air temperatures, with daily food pellets and water ad libitum. We followed Beni-Suef University’s Institutional Animal Care and Use Committee (IACUC) guidelines (BS-FS-2018-2014).

PD, Streptozotocin (STZ), and nicotinamide (NA) have been obtained as a powder from Sigma Aldrich Co. MO, USA. Metformin hydrochloride (MET) (Glucophage Xr 1000 mg) obtained from Merck KGaA, Darmstadt, Germany. Chitosan (MW medium: average molecular weight 200 kDa) was obtained from Techno Pharmchem Co. Delhi, India. PD-CSNPs were synthesized and characterized as mentioned in the recent study of Abdel-Moneim et al [20].

2.2. Induction of type 2 diabetes in rats

In cold citrate buffer (pH 4.5), streptozotocin (50 mg/kg b. wt.) [20] was dissolved and injected intraperitoneally immediately in overnight-fasted rats, after 15 min, nicotinamide (110 mg/kg b. wt. intraperitoneally) which prepared in normal physiological saline [24], then after 4 h, oral glucose load (10%) is given in water to prevent hypoglycaemia. After seven days of streptozotocin injection, fasting blood glucose concentrations were estimated using a glucometer device (CERA-CHECK™ 1070, Korea). Diabetic rats have a fasting glucose concentration of approximately 180 mg/dl have been contributed to the experiment.

2.3. Animal grouping

Experimental rats were classified into six groups, eight animals in each group; (C) normal control rats, (D) the untreated diabetic control rats, (D+CSNPs) the diabetic rats provided with nanoparticles of equivalent blank chitosan, (D + PD-CSNPs) the diabetic group-administered polydatin-loaded chitosan nanoparticles equivalent to 50 mg/kg b. wt. of polydatin [20], (D + PD) the polydatin-treated diabetic group with 50 mg/kg b. wt, and (D + MET) the metformin-HCL-treated diabetic group with 100 mg/kg b. wt [20]. After seven days of STZ injection, the doses have been given daily by gastric intubation for 1 month.

2.4. Blood sample and lung tissue assay

Snap-frozen lungs were thawed and were dissected; freshly excised lungs were weighed, minced, and homogenized in 80 mM Tris–HCl (pH 6.8). The homogenate was used to determine oxidative stress-related markers.

Lung homogenate clear supernatant was used for antioxidant enzyme levels assessment as superoxide dismutase (SOD) activity [25], catalase (CAT) activity [26], peroxidase (POX) activity [27] and reduced glutathione (GSH) concentration [28] together with lipid peroxides (MDA) content [29]. Furthermore, a blood glycosylated haemoglobin (HbA1c) percentage kit was purchased from Biosystems (Spain).

2.5. Quantitative real-time PCR

Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) has been used to test the effect of PD-CSNPs, PD, and MET on mRNA abundance of Nrf2 and haem oxygenase-1 (HO-1). Total RNA was isolated from frozen-pulmonary samples using total RNA isolation kits and quantified at 260 nm cDNAs where synthesized from 2 mg RNA and amplified using SYBR green master mix (Thermo Scientific, USA) with the primer sets Nrf2 primers (Forward: 5′-ATCCAGACAGACACAGTGGATC-3′, Reverse: 5′-GGCA GTGAAGACTGAACTTTCA-3′); HO-1 primers (Forward: 5′-TGCTCAACATCCAGCTCTTTGA-3′, Reverse: 5′-CGCTCAATTGCCGATAGTGAT-3′). The amplification data obtained were analysed using the 2−ΔΔCt method [30] and the values were normalized to β-actin and presented as % of control.

2.6. Histological and immunohistochemical studies

Lung specimens would be fixed in 10% neutral formalin buffered for 24 h, then treated by paraffin embedding method and dehydrated by ascending grades of ethyl alcohol, xylene clearing and paraffin submerged, then coated in paraffin wax at 60 °C. Sections of 4–5 μm in thickness have been dyed with haematoxylin and
recorded using image slave software (Image J) [33]. The histochemistry staining for each marker was digitally accelerated voltage 5400 LV). The intensity of immunohistology staining for each studied groups were examined by JEOL (JSM with accelerating voltage 5400 LV). The intensity of immunohistology staining for each marker was digitally recorded using image slave software (Image J) [33].

2.7. Ultrastructural preparations

Approximately 1 mm³ of pulmonary tissue was sliced, fixed in 3% glutaraldehyde-formaldehyde. The specimens washed in phosphate buffer (pH 7.4), then were post-fixed for one hour at 4°C in isoionic 1% osmium tetroxide. The electron microscopic examination prepared sections were following the Bozzola and Russell procedures [34]. Stained semi-thin sections with toluidine blue were used for detection of the area of interest, and then ultrathin sections were prepared by using the ultra-microtome glass knives. The specimens were stained by uranyl-acetate and lead-citrate and then examined by a Joel CX 100 transmission electron microscope.

2.8. Statistical analysis

Results were analysed and presented as mean ± standard error. Data were analysed using statistical package for the social sciences (SPSS) version 20 for Windows software system (SPSS Inc, Chicago, IL, USA). To compare the data between experimental groups one-way analysis of variance (ANOVA) was run and supported by the least significant difference multiple comparisons test. A simple linear correlation analysis was processed by Pearson’s method to measure the degree of dependency between variables. Differences were considered significant at \( P < 0.05. \)

3. Results

3.1. HbA1c levels

HbA1c in diabetic rats exhibited significant (\( P < 0.001 \)) increases as relative to the normal control rats, however, it was obviously diminished in diabetic rats treated with each of PD-CSNPs, PD and MET when compared to diabetic control one, whereas PD-CSNPs is more antidiabetic effect than free PD and MET (Table 1).

3.2. Pulmonary oxidative stress and antioxidants

The lung of diabetic rats revealed a significant (\( P < 0.001 \)) elevation in MDA product and reduction in antioxidants profile (GSH content, SOD, POX and CAT activities) compared to the normal control rats. These changes were ameliorated in animals treated with each of PD-CSNPs, PD and MET relative to the diabetic control rats, whereas the content of MDA product decreased and GSH content, SOD, POX and CAT activities in lung tissue increased relative to the diabetic control rats (Table 1). The ameliorative effect was more effective in animals treated with PD-CSNPs than rats treated with MET, then with PD.

3.3. Quantitative real-time PCR

The lung gene expressions (Nrf2 and HO-1) were significantly (\( P < 0.001 \)) decreased in lung tissue of diabetic animals relative to those of the normal control rats, diabetic animals treated with CSNPs showed a non-ameliorative change. While, treatment diabetic rats with PD-CSNPs, PD and MET for one month showed a significant (\( P < 0.001 \)) increase in the levels of Nrf2 and HO-1 gene expressions (Figure 1). These results illustrated that PD-CSNPs was more effective than free PD and MET in diabetic rats. Regarding correlation analysis, HbA1c revealed a positive correlation with MDA (\( r = 0.719; P < 0.001 \)) and a negative correlation with SOD (\( r = -0.734; P < 0.001 \)) and POX.

Table 1. Effect of polydatin-loaded chitosan nanoparticles, polydatin and metformin-HCL and on glycosylated haemoglobin (HbA1c), lipid peroxides (MDA), reduced glutathione concentration (GSH), superoxide dismutase activity (SOD), peroxidase activity (POX), and catalase activity (CAT) in the studied groups.

| Groups          | Tests   | C             | D              | D + CSNPs       | D + P-CSNPs     | D + PD          | D + MET        |
|-----------------|---------|---------------|----------------|----------------|----------------|----------------|---------------|
| HbA1c (%)       |         | 4.533 ± 0.169 | 7.633 ± 0.242 |
| MDA (nmol/g protein) |         | 99.291 ± 6.074 | 254.581 ± 11.779 |
| GSH (nmol/mg protein) |         | 13.418 ± 1.399 | 5.448 ± 0.364 |
| SOD activity (U/g protein) |         | 2.471 ± 0.131 | 0.723 ± 0.107 |
| POX activity (U/g protein) |         | 0.764 ± 0.028 | 0.367 ± 0.033 |
| Catalase activity (U/g protein) |         | 0.444 ± 0.083 | 0.038 ± 0.008 |

Data are expressed as mean ± SEM (n = 6). C: Control rats; D: Diabetic rats; D + CSNPs: Diabetic rats treated with blank chitosan nanoparticles; D + P-CSNPs: Diabetic rats treated with polydatin-loaded chitosan nanoparticles; D + PD: Diabetic rats treated with polydatin; D + MET: Diabetic rats treated with Metformin-HCL.

\*\*\* P < 0.001 versus control.
\* P < 0.05, \*\* P < 0.01 and \*\*\* P < 0.001 versus diabetic.
Figure 1. Effect of polydatin-loaded chitosan nanoparticles, polydatin, and metformin-HCL on lung gene expressions (Nrf2 and HO-1) in the studied groups. Data are expressed as mean ± SEM (n = 6). ###P < 0.001 versus control; ***P < 0.001 versus diabetic. C: Control rats; D: Diabetic rats; D + CSNPs: Diabetic treated with blank chitosan nanoparticles; D + PD-CSNPs: Diabetic rats treated with polydatin-loaded chitosan nanoparticles; D + PD: Diabetic rats treated with polydatin; D + MET: Diabetic rats treated with metformin-HCl.

Table 2. Correlation between HbA1c and lipid peroxidation biomarker (MDA), antioxidant enzymes (SOD, CAT, POX, GSH) and antioxidants resistance factors (Nrf2 and HO-1) in lung tissues.

| Parameters | R     | P value |
|------------|-------|---------|
| MDA        | 0.719*** | 0.001   |
| SOD        | −0.734*** | 0.001   |
| CAT        | −0.684*** | 0.001   |
| POX        | −0.734*** | 0.001   |
| GSH        | −0.728*** | 0.001   |
| Nrf2       | −0.812*** | 0.001   |
| HO-1       | −0.795*** | 0.001   |

***Correlation is significant at the 0.001 level (2-tailed). 
HbA1c: glycosylated haemoglobin; MDA: lipid peroxides; SOD: superoxide dismutase activity; CAT: catalase activity; POX: peroxidase activity; GSH: reduced glutathione concentration; Nrf2: nuclear factor erythroid 2-related factor 2; HO-1: haem oxygenase-1.

(r = −0.734; P < 0.001), GSH (r = −0.728; P < 0.001), Nrf2 (r = −0.812; P < 0.001) and HO-1 (r = −0.795; P < 0.001) in lung tissues as illustrated in Table 2.

3.4. Histopathological examination

The histological observations from normal control rats of the lung tissue showed normal lung architecture with a normal blood vessel, bronchiole, and alveoli lined mostly with squamous cells (type I pneumocyte), cuboidal cells (type II pneumocyte), and thin alveolar septum (Figure 2(a,b)). Lung sections of diabetic control rats illustrated loss of lung normal architecture (Figure 2(c,d)). Diabetic animals treated with CSNPs had no ameliorative effect on diabetic lung (Figure 2(e)). Otherwise, PD-CSNPs treatment illustrated a noticeable amelioration in the architecture of bronchiole, alveoli and blood vessel (Figure 2(f)), diabetic animals treated with PD regained the normal structure of bronchiole and alveoli with blood vessels congestion (Figure 2(g)), and treatment with MET revealed the almost normal structure of bronchiole, alveoli and blood vessel (Figure 2(h)). Masson’s trichrome stained lung sections from normal control rats showed normal very fine collagen fibres around bronchiole, blood vessels, and interalveolar septum (Figure 3(a)). The lung of diabetic control animals (Figure 3(b)) and diabetic rats treated with CSNPs (Figure 3(c)) demonstrated significant collagen fibres deposition around bronchiole, blood vessels, and interalveolar septum. Further, PD-CSNPs, PD and MET reduced the pulmonary fibrosis in diabetic rat lung tissues (Figure 3(d–f)). The histopathological examination showed that PD-CSNPs was observed marked alleviative effect against DM-induced pulmonary disorders than MET and PD.

3.5. Immunohistochemical studies

Immunohistochemical NF-κβ and TNF-α stained lung section of normal control rats showed negative expression (Figures 4 and 5(a)). The lung of diabetic control animals (Figures 4 and 5(b)) and diabetic rats treated with CSNPs (Figures 4 and 5(c)) showed positive immunoreactions in pneumocyte nuclei for NF-κβ and
Figure 2. Photomicrograph of haematoxylin and eosin stained pulmonary tissue section of (a and b): control animals showing normal alveoli (A) lined mostly with type I pneumocyte (PI) and type II pneumocyte (PII) and thin alveolar septum (arrow), bronchiole (B) and (H and E; (a) Scale bar = 200 μm and (b) Scale bar = 100 μm), (c and d): diabetic control animals displaying loss of normal architecture of the lung with thickening of interalveolar septum (arrow head), marked perivascular and peribronchiolar mononuclear cellular infiltration (IF), haemorrhage blood vessels (H), severely collapsed alveoli (ca) with compensatory dilatation of others (da), marked hyperplasia of dilated bronchiole wall (arrow), fibrosis (F) of the surrounding bronchiole and extravasation of red blood cells in the bronchiole lumen (asterisk) (H and E; (c) Scale bar = 200 μm and (d) Scale bar = 100 μm), (e): diabetic rats treated with blank chitosan nanoparticles showing collapsed alveoli (ca), dilatation of neighbouring ones (da), peribronchiolar mononuclear cellular infiltration (IF), haemorrhage (H) and fibrosis (arrow) of the surrounding bronchiole, (f): diabetic group treated with polydatin-loaded chitosan nanoparticles illustrating a marked improvement in the structure of bronchiole (B), alveoli (A) and blood vessel (arrow), (g): diabetic group treated with polydatin regained the normal structure of bronchiole (B) and alveoli (A) with congestion of blood vessels (arrows), (h): diabetic group treated with metformin-HCL showing almost normal structure of bronchiole (B), alveoli (A) and blood vessel (arrow) (H and E; Scale bar = 200 μm).
Figure 3. Photomicrograph of masson’s trichrome stained pulmonary tissue section of (a): control rats showing normal distribution of collagen fibres around bronchiole (B), blood vessels (BV) and interalveolar septum (arrow), (b): diabetic control animals indicating severe deposition of blue collagen fibres around bronchiole (B), blood vessels (BV) and interalveolar septum (arrow), (c): diabetic rats treated with blank chitosan nanoparticles showing deposition of blue collagenous fibres around bronchiole, blood vessels and interalveolar septum (arrow), (d): diabetic group treated with polydatin-loaded chitosan nanoparticles showing disappearance of collagen fibres around bronchiole and interalveolar septum (arrow), (e): diabetic group treated with polydatin illustrating decreasing of collagen fibres around bronchiole (B), interalveolar septum (arrow) and blood vessels (BV), (f): diabetic group treated with metformin-HCL showing a marked amelioration including a decrease in collagen fibres around bronchiole (B), blood vessels (BV) and interalveolar septum (arrow) (Masson’s trichrome; Scale bar = 200 μm).
Figure 4. Photomicrograph of nuclear factor-kappa β (NF-κ β) expression stained pulmonary tissue section (a): normal control rats showing a negative reaction, (b): diabetic control rats showing intense brown positive immunoreaction in nuclei of pneumocytes (arrow), (c): diabetic rats treated with blank chitosan nanoparticles showing immune-positive reaction in nuclei (arrow), (d): diabetic group treated with polydatin-loaded chitosan nanoparticles showing negative immunoreaction, (e): diabetic group treated with polydatin showing weak immunoreaction, (f): diabetic group treated with metformin-HCL showing weak immunoreaction (NF-κ β; Scale bar = 50 μm).
Figure 5. Photomicrograph of tumour necrosis factor-alpha (TNF-α) expression stained pulmonary tissue section of (a): normal control rats showing a negative reaction, (b): diabetic control rats showing intense brown immunoreaction in cytoplasm of pneumocytes (arrow), (c): diabetic rats treated with blank chitosan nanoparticles showing immune-positive reaction in cytoplasm (arrow), (d): diabetic group treated with polydatin-loaded chitosan nanoparticles showing negative immunoreaction, (e): diabetic group treated with polydatin showing weak immunoreaction, (f): diabetic group treated with metformin-HCL showing weak immunoreaction (TNF-α; Scale bar = 50 μm).
plemocyte cytoplasm for TNF-α, respectively. Also, both NF-κβ and TNF-α showed a significant intensity in immunohistochemistry staining than that of normal control rats (Table 3). PD-CSNPs displayed negative nucleic immunoreactions for NF-κβ and negative cytoplasmic immunoreactions for TNF-α, respectively (Figures 4 and 5(d)), where PD (Figures 4 and 5(e)) and κβ and TNF- for NF-κβ cytoplasmic immunoreactions for TNF-α of PD-CSNPs relative to MET and PD.

bodies, microvilli and mitochondria (Figure 6(h)). The

6(g)), while treatment with MET revealed nearly nor-

mals with PD showed improvement in the nucleus, lamellar bodies, microvilli, and mitochondria (Figure 6(f)). Treatment of diabetic rats with chitosan nanoparticles showed no ameliorative effects in diabetic control rats (Table 3). The data showed that the ameliorative effect of PD-CSNPs was more effective than MET and PD.

3.6. Ultrastructural studies

Ultrastructural observations of normal control lung tissue showed normal alveolar vector with type II pneumocyte had a prominent nucleus, many electron-dense lamellar bodies, microvilli, and mitochondria (Figure 6(a)). Lung sections of diabetic control rats displayed severe degenerative type II pneumocyte with marked loss of most cytoplasmic components, lamellar bodies nearly devoid of secretory lamellated material, lack of microvilli along the margin of the alveolar type II pneumocyte, irregular nucleus, marked deposition of collagen fibres and inflammatory cell infiltration (Figure 6(b,c)). Treatment of diabetic rats with chitosan nanoparticles showed no ameliorative effects in diabetic rats (Figure 6(d,e)). However, treatment with PD-CSNPs demonstrated marked improvement reflected by a regular nucleus, lamellar bodies, microvilli, and mitochondria (Figure 6(f)). Treatment of diabetic animals with PD showed improvement in the nucleus, lamellar bodies, microvilli, and mitochondria (Figure 6(g)), while treatment with MET revealed nearly normal type II pneumocyte with a regular nucleus, lamellar bodies, microvilli and mitochondria (Figure 6(h)). The ultrastructural studies confirmed the ameliorative effect of PD-CSNPs relative to MET and PD.

4. Discussion

 Chronic exposure to high blood glucose levels in DM resulting in the development of bronchial tree fibrotic tissue that may cause complications in the lung of diabetic patients [35]. The main goal in diabetes management is the prevention of DM-induced pulmonary disorders to maintain normal blood glucose levels and redox state. The present results suggested that by exhibiting antidiabetic activity, oral administration of PD-CSNPs, PD and MET individually for one month significantly reduced HbA1c % compared to diabetic control rats. The present findings agree with reports showed that the hypoglycaemic effects of PD-CSNPs [20], PD [19] and MET [36] were evident.

Several hyperglycaemia-upregulated pathways can potentially include diabetic lung injury, including oxidative stress, the pathway of the inflammatory responses, NF-κβ, and mitochondrial disorder [37]. Oxidative stress is a serious pathological pathway for T2DM [38], which increase cell stress and lead to a reduction in antioxidant intracellular defenses. Furthermore, MDA has been obviously elevated [39] and enzymatic antioxidants SOD, CAT, GSH were significantly lower in diabetic animals [40]. Inflammatory and apoptotic markers including NF-κβ, TNF α, Nrf2, and HO-1 protein, and gene expression were induced after the production of mitochondrial ROS caused by oxidative damage [41]. Additionally, NF-κβ is a protein–DNA binding factor involved in the transcription of various proinflammatory and inflammatory molecules such as cytokines, chemokines, cell adhesion molecules (CAM), and various enzymes [42]. Active Nrf2 modulates the expression of the antioxidant proteins that defend against injury and oxidative damage associated with inflammation [43], inhibits NF-κβ activation by inducing numerous cytoprotective proteins such as HO-1 [44]. The present study revealed that hyperglycaemia was associated with Nrf2 and HO-1 signaling pathway dysfunction and reported positive NF-κβ immunoreactions in the pneumocyte nuclei and positive TNF-α immunoreactions in the pneumocyte cytoplasm in the diabetic animals.

The current investigation elucidated that oral treatment of PD-CSNPs, PD and MET reduced lipid peroxidation products and increased the enzymatic antioxidant activity; also exhibited their anti-inflammatory effects and suppressed inflammatory damage in diabetic rats through enhancing expression of Nrf2 and HO-1, which reduces oxidative stress. Notably, polydatin
Figure 6. Electron micrograph of lung section (a): normal control rats alveolar tissue showing type II pneumocyte with prominent nucleus (N), many electron-dense lamellar bodies (L), microvilli (arrow head) and mitochondria (arrow), (b and c): diabetic control rats showing severe degenerative type II pneumocyte with marked loss of most cytoplasmic components (arrow), lamellar bodies nearly devoid of secretory lamellated material (L), lack of microvilli along the alveolar type II pneumocyte margin, irregular nucleus (N) and marked deposition of collagen fibres (CF); Inset: showing inflammatory cell infiltration (arrow head), (d and e): diabetic rats treated with blank chitosan nanoparticles showing marked degenerative type II pneumocyte (asterisk), pyknotic nucleus (N), few scattered microvilli (arrow head), irregular arranged lamellar bodies (L), collagen fibres (CF) and alveolar macrophage (M) with cytoplasmic lysosomes (arrow), (f): diabetic rats treated with polydatin-loaded chitosan nanoparticles showing marked improvement represented by nucleus (N), lamellar bodies (L), microvilli (arrow) and mitochondria (M), (g): diabetic rats treated with polydatin showing improvement in nucleus (N), lamellar bodies (L), microvilli (arrow) and mitochondria (M), (h): diabetic rats treated with metformin-HCL showing the type II pneumocyte is more similar to the normal control group with regular nucleus (N), lamellar bodies (L), microvilli (arrow) and mitochondria (M) (Scale bar = 2 μm).
nanoparticles revealed anti-lipid peroxidation and antioxidant activity [45]. Also, PD reduced hyperosmolar stress-induced inflammation by attenuating NF-κB translocation to the nucleus and the TNF-α mRNA expression [16]. Interestingly, MET treatment reduced the numbers of oxidative stress factors by controlling the antioxidant system of the cells [23].

Histopathological examinations of the lung supported the observed biochemical and immunohistochemical findings, whereas, the diabetic lung showed inflammatory and fibrotic changes that are in parallel with the studies of Talakatta et al. [9]. Importantly, the present study showed that treatment with PD-CSNPs, PD and MET has preserved the diabetic rat lung architecture. This could be attributed to their roles as antioxidants, anti-inflammatory, and anti-apoptotic functions. PD administration inhibited inflammatory responses and progression of pulmonary fibrosis through suppressing the NF-κB pathway [46] and MET inhibit the inflammatory processes and oxidative stress [23].

Diabetes caused the type II pneumocyte with marked loss of most cytoplasmic components, lamellar bodies nearly devoid of secretory lamellated material, lack microvilli along the margin of the alveolar type II pneumocyte, irregular nucleus, marked deposition of collagen fibres and inflammatory cell infiltration compared with those of normal control rats. These alterations showed an increased membrane thickness that can inhibit the transportation of gases by diffusion and lead to a reduced supply of oxygen to the tissue [47]. Treatment with PD-CSNPs, PD, and MET showed noticeable preventive effects against ultrastructural alterations in lung of diabetic rats. Whereas, PD may be a possible therapeutic candidate in the treatment of pulmonary fibrosis [46] and MET is successful in the treatment of chronic obstructive pulmonary disease in patients with concomitant T2DM [48]. The used synthetic agents for the treatment of diabetes have limitations because of inadequate efficiency, high cost, and side effects such as hypoglycaemia, weight gain, gastrointestinal disturbances, and liver toxicity [49]. Whereas this study revealed that the new formula of PD-CSNPs was more effective than MET and free PD in lung protection against DM-induced pulmonary disorders. PD has low bioavailability because of poor aqueous solubility, chemical instability in aqueous alkaline medium [50]. The correlation analysis for alterations in lung tissues revealed a significant positive correlation between the elevation of HbA1c% and MDA in lung and HbA1c% recorded a marked negative correlation with the levels of antioxidant system e.g. SOD, CAT, GPX, GSH, Nrf2 and HO-1. The present study showed that PD-CSNPs have many potential activities and practical advantages relative to MET and free PD, especially against oxidative damage-induced pulmonary disorders associated with diabetes. PD-CSNPs can be optimized at the molecular level to make them more effective and may be beneficial as antidiabetic agents. However, pharmacokinetic analyses of the current PD-CSNP formula are needed prior to clinical trials to determine the balance between the effectiveness and toxicity of the new therapeutic agents which could be useful as an alternative to synthetic drugs.

5. Conclusion
PD-CSNP new formula exhibited a significant antidiabetic efficacy relative to free PD and MET since PD-CSNPs was a potential nanocarrier for sustained PD delivery. PD-CSNPs, therefore, was more effective in lung protection against DM-induced pulmonary disorders in diabetic rats through reducing lung levels of TNF α and NF-κβ and promoting Nrf2/HO-1 signaling. PD-CSNPs also mitigate markedly oxidative stress biomarkers, histopathological and ultrastructural alterations in diabetic rats than the effect of MET and PD.

Disclosure statement
No potential conflict of interest was reported by the authors(-).

ORCID
Fatma Mostafa http://orcid.org/0000-0003-3274-6395
Hanaa M. Mohamed http://orcid.org/0000-0001-6252-2240
Adel Abdel-Moneim http://orcid.org/0000-0002-1254-0894
Manal Abdul-Hamid http://orcid.org/0000-0002-0877-3097

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