Archives of Pharmaceutical Sciences Ain Shams University 2025; Vol. 9(2): 300-313

OPEN ACCESS

Pharmacology and Toxicology

Original Article

Exploring the protective effects of chrysin against bleomycin-induced lung injury and fibrosis: The role of autophagy and IL-33/ST2

Marwa Nabil[a*], Haidy E. Michel[b], Esther T. Menze[b], Mohamed F. Abd-Ellah[c], Ebtehal El-Demerdash[b]

[a]*Egypt Ministry of Health and Population, Cairo, Egypt*
[b]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt*
[c]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt*

ABSTRACT

Bleomycin is an effective antineoplastic agent. However, it induces lung fibrosis. Chrysin, a natural flavone, possesses multiple biological activities, such as antioxidant, anti-inflammatory, and anti-cancer. This study investigated whether chrysin could protect against the devastating effects of bleomycin on lung tissues and the underlying mechanism of this effect. Rats were treated with either bleomycin (15 mg/kg, i.p., 3 times per week for 4 weeks) and/or chrysin (50 mg/kg daily, starting one week before bleomycin administration and until the end of the experiment). Markers of lung injury, oxidative stress, inflammation, autophagy, and the IL33/ST2 signaling pathway were assessed. Bleomycin significantly increased the levels of lactate dehydrogenase (LDH) and lipid peroxidation, and it significantly decreased mucin and glutathione compared to the control group. These effects were ameliorated by chrysin treatment. Histopathological analysis found evidence of severe pulmonary inflammation and increased levels of the inflammatory mediators, tumor necrosis factor-alpha, interleukin-6, and nuclear factor kappa-B (NF-κB) (p65). Fibrosis was evident by the increase in α-smooth muscle actin and transforming growth factor-β1 (TGF-β1). Inflammation and fibrosis were reduced in rats that received the chrysin treatment concurrent with bleomycin. In addition, autophagy was inhibited in the lung tissues of bleomycin-treated rats, as evidenced by the reduced expression of ATG4B and LC3. Furthermore, the IL-33/ST2 signaling pathway was activated by bleomycin, and this effect was markedly attenuated by chrysin treatment. In conclusion, chrysin mitigated bleomycin-induced pulmonary fibrosis in rats through its antioxidant and anti-inflammatory effects, as well as having an effect on autophagy and the IL-33/ST2 signaling pathway.

Keywords: *Chrysin; bleomycin; autophagy; IL33/ST2; pulmonary fibrosis*.

***Correspondence** | Marwa Nabil; Egypt Ministry of Health and Population, Cairo, Egypt. Email: marwanabeel@outlook.com

Citation | Nabil M, Michel HE, Menze ET, Abd-Ellah MF, El-Demerdash E, 2025. Exploring the protective effects of chrysin against bleomycin-induced lung injury and fibrosis: The role of autophagy and IL-33/ST2. Arch Pharm Sci ASU 9(2): 300-313
DOI: 10.21608/aps.2025.354713.1215
Print ISSN: 2356-8380. **Online ISSN**: 2356-8399.
Received 29 January 2025. **Accepted** 24 March 2025.

Published by: Ain Shams University, Faculty of Pharmacy

1. Introduction

Pulmonary fibrosis is caused by many factors. Its characteristics include fibroblast production as well as extracellular matrix remodeling. Most cases of pulmonary fibrosis are idiopathic (IPF). IPF is a progressive lung disease of unknown etiology, accompanied by abnormal extracellular collagen deposition **[1]**. It is characterized by the recruitment of macrophages and neutrophils in the alveoli, the release of large amounts of reactive oxygen species (ROS) leading to lung disease, and the generation of fibroblasts in the alveolar wall **[2]**. Therefore, it is hypothesized that IPF-related injury of alveolar epithelial cells might be due to elevated oxidative

stress in the lower respiratory tract. Unfortunately, IPF has a poor prognosis, and despite persistent research, there are no effective treatment options available **[3]**.

Interleukin-33 (IL-33) is a member of the interleukin-1 (IL-1) cytokine family, and it is an extracellular ligand for the IL-1 receptor, ST2. The IL-33/ST2 axis plays an essential role in immunity and cellular homeostasis by promoting wound healing and tissue repair. It is also implicated in an imbalance between excessive inflammation and tissue remodeling, causing remodeling, the underlying key to fibrosis **[4, 5]**. In addition, research has shown that IL-33 plays an important role in the regulation of autophagy, as well as tissue fibrosis **[5]**.

Bleomycin (BLM) is an antineoplastic antibiotic used to treat certain cancers, such as lymphoma, head and neck squamous cell carcinoma, testicular carcinoma, and ovarian cancer **[6]**. BLM-induced pulmonary fibrosis is commonly used as an animal model of human IPF. It induces an inflammatory response, alveolar cell injury, fibroblast accumulation, and collagen deposition in rodents **[7, 8]**.

Chrysin (5.7-dihydroxyflavone) is a flavone found in propolis, honey, and various plants. In addition, it was extracted from passionflower **[9]**. It is a secondary metabolite produced by plants as a first line of defense against UV radiation, the production of oxygen radicals, and/or invading organisms **[10]**. It has many biological activities, such as antioxidant, anti-inflammatory, lipid-lowering, and hypoglycemic effects **[11,12]**. In addition, chrysin can reduce pulmonary fibrosis caused by BLM. The method by which chrysin can improve fibrosis can be described by its anti-inflammatory, antioxidant, and anti-fibrotic effects as well as its ability to reduce hypoxia **[11]**. The present study aimed to elucidate the protective mechanisms of chrysin against BLM-induced pulmonary fibrosis by exploring its influence on autophagy and the IL33/ST2 signaling pathway.

2. Materials and Methods

2.1. Drugs and chemicals

Bleomycin ampoules were purchased (Bleocip®) from Cipla Company (Mumbai, India). Each ampoule contains 15 mg of BLM HCl in powder form. The powder is white and soluble in saline. Chrysin was purchased from Alfa Aesar (Lancashire, UK). Chrysin is dissolved in corn oil, and to ensure a homogeneous suspension, it is carefully vortexed just before administration. Other reagents were of the highest purity available on the market.

2.2. Animals

Forty male Sprague Dawley rats (200–250 g) were purchased from Nile Company, EL-Amyria, Cairo, Egypt. Plastic cages were used as their habitat at a constant temperature (21 ± 2 °C) for a 12-h light/dark cycle. All rats have free access to food and water. The animals had a week of acclimatization to laboratory conditions before starting the experiment. Animal treatments are strictly regulated according to the National Research Council Guidelines for the Care and Use of Laboratory Animals.

2.3. Experimental design

The rats were divided into four groups of ten each. The first group was the control group, which received the corresponding saline vehicles. The second group received chrysin (50 mg/kg, p.o.) daily for 35 days **[13]**. The third group received BLM (15 mg/kg, i.p.) three times a week for a total of four weeks **[14]**. The fourth group received chrysin (50 mg/kg, p.o.) daily for 35 days, beginning one week before receiving BLM (15 mg/kg, i.p.), and given three times a week for a total of four weeks, simultaneously with chrysin.

Twenty-four hours after the last chrysin dose,

the rats were euthanized by rapid cervical dislocation under thiopental anesthesia (50 mg/kg, i.p.). After lung and tracheal dissection, three consecutive doses of 7 mL of 0.9% saline solution were infused through a catheter inserted into the trachea and then slowly withdrawn from the lungs. This bronchoalveolar lavage (BALF) was centrifuged at 500 × g for 10 min at 4 °C. The supernatant was then isolated and stored at -80 °C until used for assessment of LDH activity, mucus concentration, and protein content. Lung tissue sections from different groups were fixed in 10% buffered formalin, and the remaining lung tissue was stored at -80 °C for further assessment of oxidative stress, inflammation, fibrosis, and autophagy markers.

2.4. Assessment of lung injury markers

Lung indices were calculated as (lung weight/body weight) x 100. LDH activity was determined colorimetrically using an assay kit (catalog number E-BC-K046-M; Elabscience Biotechnology Inc., Texas, USA). BALF protein content was determined at 595 nm using a commercial Bradford colorimetric kit (catalog number QCPR-500; BioAssay Systems, 3191 Corporate Place, Hayward, CA 94545, USA). The mucin content was determined using a rat-specific enzyme–linked immunosorbent assay (ELISA) kit (catalog number MBS7201306; MYBiosource, San Diego, USA, USA).

2.5. Histopathological examination

Six lung samples were washed, dehydrated with alcohol, cleared with xylene, and then dipped in paraffin at 56 °C in a hot-air oven for 24 h. Next, hematoxylin and eosin (H&E) dyes were used to stain the samples, followed by histological evaluation by light microscopy **[15]**.

2.6. Assessment of oxidative stress markers

Glutathione (GSH) and lipid peroxidation were determined in accordance with the kits' instructions (catalog numbers GR 25 10 and MD 25 28; Biodiagnostics, Giza, Egypt). GSH was determined using the method described by **[16]**. Lipid peroxidation was determined by estimating the level of malonaldehyde (MDA) using thiobarbituric acid **[17]**.

2.7. Assessment of inflammatory markers

Interleukin-6 (IL-6) and TNF-α were determined using ELISA kits purchased from R&D Systems, Inc. (Minneapolis, USA) and CUSABIO (Houston, USA), with catalog numbers R6000B and CSB-E11987r, respectively. The assays were conducted according to the manufacturer's protocols. NF-κB was determined using RNA extraction and real-time PCR analysis. From tissue homogenization, total RNA was extracted using the SV Total RNA Isolation system (Thermo Scientific, USA). Quantitative real-time one-step PCR was performed using QuantiTect® SYBR® Green RT-PCR master mix kit (Qiagen, Hilden, Germany). Relative mRNA expression of target genes was measured by normalizing against the control group; the $2^{-\Delta\Delta Ct}$ method was used to establish the level of β-actin. The primer sequences of β-actin and NF-κB **[18]** are shown in **Table 1**.

Table 1. Primers Sequence of beta actin and NF-κB (18)

Gene	Forward primer	Reverse primer
NF-κB	CATGAAGAGAAGACACTGACCATGGAAA	TGGATAGAGGCTAAGTGTAGACACG
β-actin	GAGACCTTCAACACCCCAGC	ATGTCACGCACGATTTCCC

In addition, IL33, CASP1, and ST2 were determined using western blot analysis. Total protein was extracted from frozen lung samples using RIPA lysis buffer (Bio Basic Inc., Ontario, Canada). Protein was resolved using 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Bio-Rad Laboratories, California, USA, catalog number: 1610181). Then, the gel was electro-transferred onto a polyvinylidene difluoride (PVDF) membrane. Membranes were blocked with Tris-buffered saline and Tween 20 (TBST) buffer and 3% bovine serum albumin at room temperature for 1 h, and incubated overnight at 4 °C with the primary antibodies, anti-IL33, anti-CASP1 and anti-ST2 at a ratio of 1:2000, (Thermo Fisher Scientific, UK, with catalog numbers: PA5-23150, PA5-29342 and PA5-23316, respectively). After overnight incubation, membranes were washed and then incubated with goat anti-rabbit IgG HRP-conjugated secondary antibody (Novus Biologicals). Development was performed using an enhanced chemiluminescence kit (ClarityTM Western ECL substrate - BIO-RAD, USA, catalog number 170-5060). ImageJ software (version 1.48) was used to determine the band intensity of the target proteins against the control sample after normalization to β-actin.

2.8. Assessment of fibrosis markers

Alpha smooth muscle actin (ASMA), collagen, and TGF-β were determined in lung tissue using rat-specific ELISA kits (MyBiosource, San Diego, USA, with catalog numbers MBS266620, MBS262647, and MBS824788, respectively). The assays were conducted according to the manufacturer's protocol.

2.9. Assessment of autophagy markers

ATG4B and LC3B were determined using western blot analysis as discussed previously. The primary antibodies used in analysis were anti-ATG4B and anti-LC3B (Thermo Fisher Scientific, UK, with catalog numbers: PA5-86908 and PA5-22940).

2.10. Statistical analysis

Data are presented as mean ± SD. One-way analysis of variance (ANOVA), followed by Tukey's test as a post hoc test, was used to detect significant differences between all experimental groups. GraphPad Prism software was used to perform all statistical analyses (version 5.01, Inc., 2007, San Diego, USA). Probability values less than 0.05 are considered to be statistically significant.

3. Results

3.1. Effect of BLM and/or chrysin treatment on lung injury markers

Compared to the control group, rats exposed to BLM showed a 35% decrease in the quantity of mucin expressed, and LDH activity increased by 116% and protein content rose by 64%. On the other hand, chrysin pretreatment restored the mucin content to normal levels. In addition, LDH activity and protein content were reduced by 45% and 21%, respectively, by chrysin pretreatment compared to the BLM group **(Table 2)**.

Table 2. Effect of chrysin on lung injury markers in BLM-treated rats

	Mucin (ng/mL)	**LDH (µmol/mL)**	**Protein (mg/mL)**
Control	304.3 ± 15.06	111.98 ± 3.3	86.6 ± 3.6
Chrysin	320.2 ± 13.48	95.88 ± 10.06	99.66 ± 6.7
BLM	199.04 ± 13.7$^{(a)}$	241.7 ± 34.29$^{(a)}$	141.72 ± 16.13$^{(a)}$
BLM + Chrysin	303.38 ± 10.3$^{(a'b)}$	133.84 ± 15$^{(a'b)}$	112.44 ± 3.69$^{(a'b)}$

3.2. Histopathological examination

Histological examination of different pulmonary tissue samples is illustrated in **Fig. 1**. The pulmonary bronchioles and vasculature of the control group and chrysin-only treatment group were normal and exhibited normal morphologies. Almost intact alveoli, including interalveolar walls, were detected in these groups **(Fig. 1A and 1B)**. In contrast, the BLM group showed marked diffuse interstitial pneumonia with significantly thickened interalveolar walls. In addition, marked peribronchiolar fibrosis with many proliferating fibroblasts, which was accompanied by inflammatory cells, was detected in this group **(Fig. 1C)**. A moderate protective effect was detected in the BLM and chrysin-treated group. Namely, persistent records of interstitial and peribronchiolar inflammatory cells infiltrates, and mild hyperplasia of the lining bronchiolar epithelium **(Fig. 1D)**.

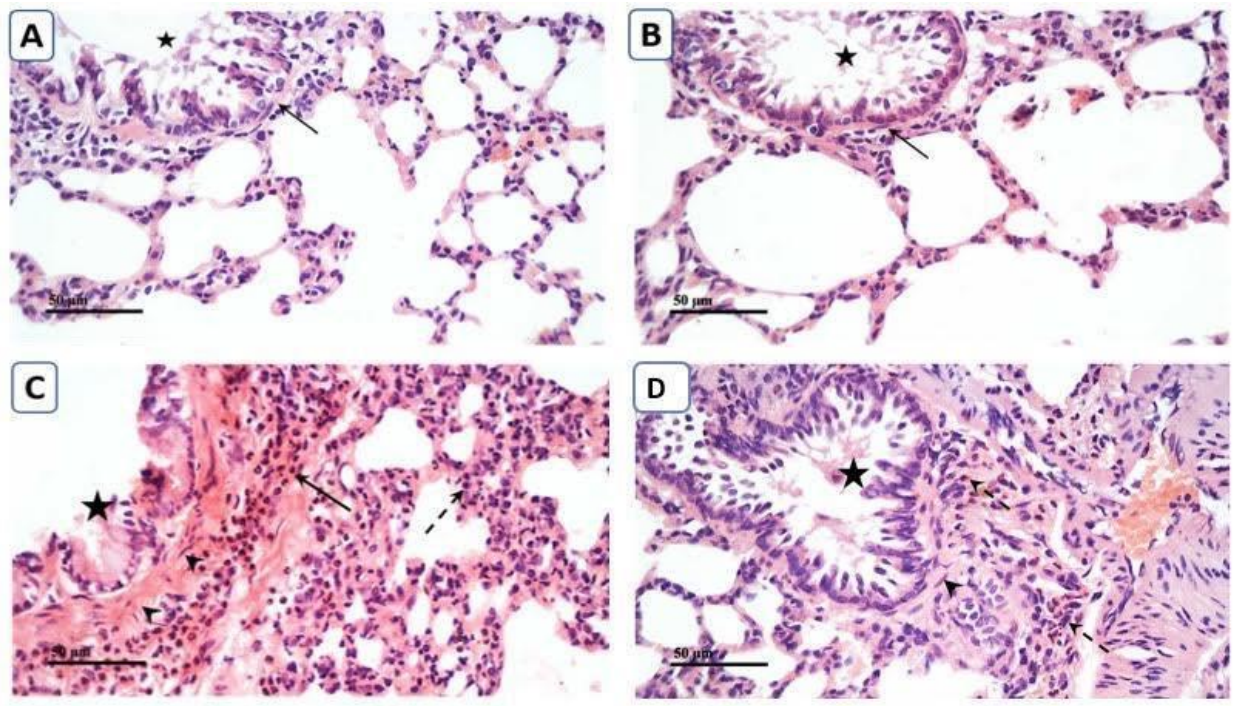

Fig. 1. Representative photomicrographs of H & E-stained lung sections (×400), **(A)** Control group, **(B)** Chrysin group, **(C)** BLM group, **(D)** BLM + Chrysin group. The control group exhibited normal morphological features of pulmonary bronchioles **(star),** surrounded by a delicate layer of connective tissue fibers and smooth muscles **(arrow)**, solitary lymphoid follicles, as well as normal vasculature. Almost intact alveoli including interalveolar walls were detected **(Fig. 1A)**. Chrysin (50 mg/kg)-treated group showed normal histological structures of pulmonary tissues with few scattered interstitial inflammatory cells without significant abnormal tissue alterations records **(Fig. 1B).** BLM-treated group showed marked diffuse interstitial pneumonia with significant increased thickness of interalveolar walls and evident mixed populations of inflammatory cells infiltrates **(dashed arrow).** Besides, a mild hyperplasia of the lining bronchiolar epithelium with mucosal infoldings was detected**.** In addition, marked peribronchiolar fibrosis with many proliferating fibroblasts **(arrow head)** accompanied with inflammatory cells infiltrations and nodular hyperplasia of associated lymphatic tissue were detected **(Fig.1C).** BLM and chrysin (50 mg/kg)-treated group showed moderate protective effect; namely, persistent records of interstitial and peribronchiolar inflammatory cells infiltrates**,** mild hyperplasia of lining bronchiolar epithelium**.** However, peribronchiolar fibrous tissue deposition was significantly minimized compared with BLM-treated animals **(Fig.1D).**

3.3. Effect of BLM and/or chrysin treatment on oxidative stress markers

The MDA level in the BLM group had risen dramatically (401%) compared to the control group; the rise was accompanied by a significant decline (68.6%) in GSH. Compared to the BLM group, pretreatment with chrysin significantly decreased MDA level by 72% and increased GSH level by 205% **(Table 3)**.

Table 3. Effect of chrysin on MDA, GSH, TNF-α, IL-6, and NFκB (P65)

	MDA (mmol/g tissue)	GSH (mmol/g tissue)	TNF-alpha (ng/g tissue)	IL-6 (pg/mL)	NF-kB (mRNA expression level)
Control	13.3 ± 2.4	69.1 ± 10.8	30.78 ± 4.4	38.10 ± 6.2	1.004 ± 0.009
Chrysin	5.804 ± 0.8	85.96 ± 4.0	18.44 ± 3.8	26.56 ± 3.9	0.89 ± 0.040
BLM	66.56 ± 16.2[(a)]	21.7 ± 3.6[(a)]	113.16 ± 21.3[(a)]	133.68 ± 15.9[(a)]	7.602 ± 1.75[(a)]
BLM + chrysin	21.48 ± 4.2[(b)]	66.26 ± 5.9[(b)]	40.14 ± 7.0[(b)]	49.66 ± 8.5[(b)]	2.042 ± 0.38[(b)]

Data are presented as means ± SD (n = 5), a, b at $P < 0.05$, statistically significant compared with the control and BLM-administered groups, respectively (one-way ANOVA followed by Tukey's test).

3.4 Effect of BLM and/or chrysin treatment on inflammation markers

Rats exposed to BLM showed significant elevations in TNF-α and IL-6 levels, 268% and 251%, respectively, compared to the control group. However, pretreatment with chrysin significantly decreased TNF-α and IL-6 levels by 64.5% and 62.9%, respectively, compared to the BLM group. In addition, BLM caused NF-κB (P65) expression to rise by 657%, compared to the control group. Pretreatment with chrysin significantly decreased NF-κB expression by 73%, compared to the BLM group **(Table 3)**.

Furthermore, compared to the control group, the expression of Casp-1, IL33, and ST2 was elevated in the BLM group by 62%, 65%, and 66%, respectively. Pretreatment with chrysin resulted in reduced expression of Casp-1, IL33, and ST2 by 62%, 64.5% and 66.5%, respectively, compared to the BLM group **(Fig. 2)**.

3.5. Effect of BLM and/or chrysin treatment on fibrosis markers

BLM caused collagen, ASMA, and TGF-β levels to increase by 402%, 254%, and 262%, respectively, compared to the control group. Pretreatment with chrysin significantly counteracted the fibrotic effect of BLM compared to the BLM group, as evidenced by the decrease in collagen, ASMA, and TGF-β levels by 59%, 25%, and 69%, respectively **(Fig. 3)**.

3.6. Effect of BLM and/or chrysin treatment on autophagy markers

The expression of ATG4B and LC3 in rats exposed to BLM declined by 78% and 86%, respectively, compared to the control group. Pretreatment with chrysin significantly increased ATG4B and LC3 expression by 285% and 536%, respectively, compared to the BLM group **(Fig. 4)**.

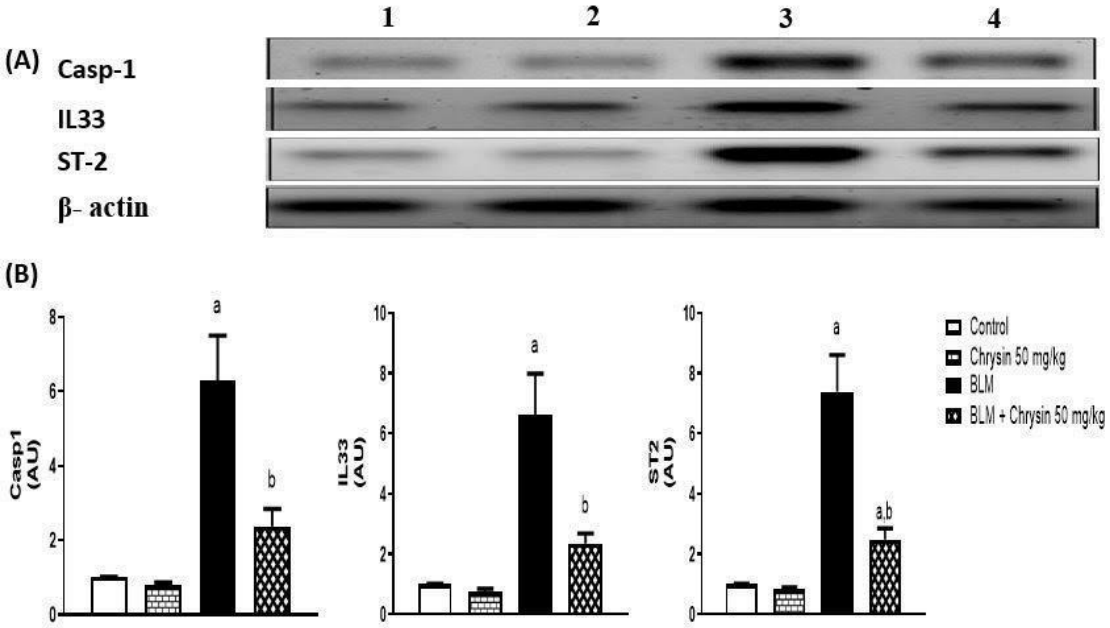

Fig. 2. Effect of chrysin and/or bleomycin treatment on A. Western blot analysis of Casp-1, IL33, and ST2 expressions. (1) control group, (2) Chrysin group, (3) BLM group, (4) BLM + Chrysin group. B. Densitometric quantitation of Casp-1, IL33, and ST2 expressions. Data are expressed as mean ± SD (n = 5) using one-way ANOVA followed by Tukey's test for multiple comparisons between groups. Where the levels of significance are $P < 0.05$. **a:** statistically significant compared to the control. **b:** statistically significant compared to the BLM group.

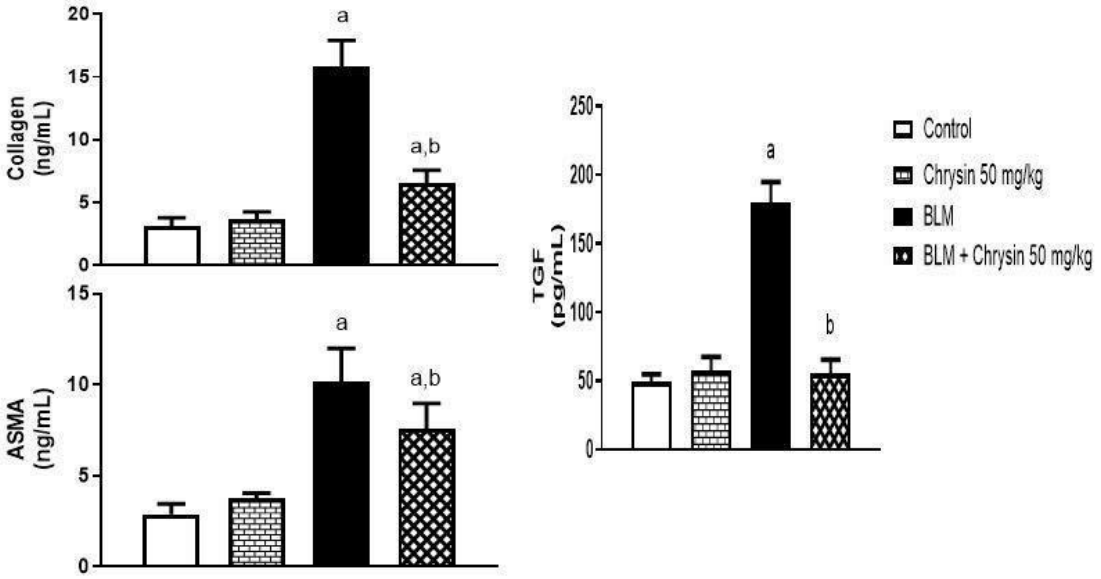

Fig. 3. Effect of chrysin and/or bleomycin treatment on fibrosis markers: collagen, ASMA, and TGF-β1. Data are expressed as means ± SD (n=5) using one-way ANOVA followed by Tukey's test for multiple comparisons between groups. Where the levels of significance are $P < 0.05$. **a:** statistically significant compared to the control. **b:** statistically significant compared to the BLM group.

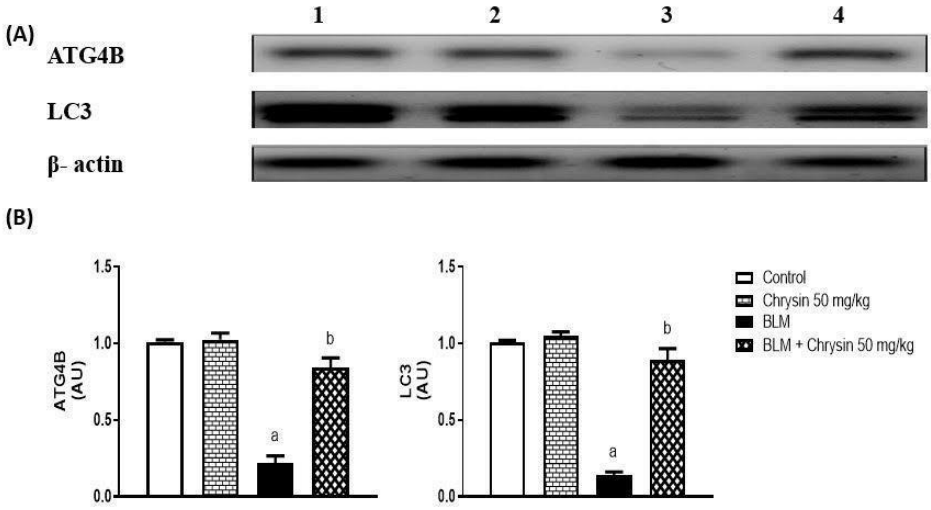

Fig. 4. Effect of chrysin and/or bleomycin treatment on A. Western blot analysis of ATG4B and LC3 expressions. 1) control group (2) Chrysin group (3) BLM group (4) BLM + Chrysin group. B. Densitometric quantitation of ATG4B and LC3 expressions. Data are expressed as means ± SD (n = 5) using one-way ANOVA followed by Tukey's test for multiple comparisons between groups. Where the levels of significance are $P < 0.05$. **a:** statistically significant compared to the control. **b:** statistically significant compared to the BLM group.

4. Discussion

Lung fibrosis induced by BLM during chemotherapy highlights the importance of finding new treatments devoid of such an effect. Accordingly, our study aimed to investigate the extent to which chrysin can counter the devastating actions of BLM and underline the possible mechanisms by which chrysin could exert protective action. To do this, we investigated different markers of lung injury, oxidative stress, inflammation, fibrosis, and autophagy. In the beginning, animals exposed to BLM alone showed a significant elevation in the biomarkers involved in lung injury, such as LDH and total protein **[19]**. There was also a significant reduction in mucin compared to the control group, while pretreatment with chrysin counteracted the effect of BLM, as all the injury markers were almost normal. Similarly, the study conducted by El-Khouly et al. **[20]** demonstrated the toxic effect of BLM in rats, with emphysema being detected in air alveoli, as well as inflammatory cells, and lymphoid hyperplastic cells; pretreatment with chrysin significantly ameliorated these pathological changes. These findings reflect the potential protective effect of chrysin against BLM-induced lung injury **[21]**. Previous studies endorsed the cytoprotective effect of chrysin in decreasing LDH, which manifested as improved lung function in cases of pulmonary hypertension and reduced edema **[22, 23]**.

One of the mechanisms involved in BLM-induced lung injury is the drug's ability to generate intracellular ROS. BLM triggers the formation of free radical species, such as superoxide anion and hydroxyl radicals **[24]**. The generation of ROS can induce the depletion of reduced GSH and the elevation of MDA, a marker of lipid peroxidation **[25]**. In the present study, significantly elevated MDA with low GSH was detected after the administration of BLM. This is consistent with other studies where BLM-induced ROS in lung tissues resulted in GSH-induced DNA stress **[26]**, mitochondrial leakage, and apoptosis **[27]**. These changes were

ameliorated by pretreatment with chrysin, which is consistent with the findings of Kseibati et al. **[19]**. That study revealed that daily oral treatment with chrysin for six weeks reduced the MDA content in lung cells by 57.5%, whilst concurrently elevating GSH by 67.6% compared to the BLM group. Similarly, a study by Hanedan and colleagues **[28]** investigated the effect of chrysin with hesperidin on renal injury induced by colistin in rats. They revealed a decrease in MDA and inflammatory parameters, together with a significant elevation in GSH, superoxide dismutase, catalase, and glutathione peroxidase, which indicate the antioxidant protective effect of chrysin. Furthermore, the antioxidant properties of chrysin were demonstrated against doxorubicin-induced cardiotoxicity **[29]**. Thus, one of the protective mechanisms by which chrysin could counteract the BLM effect is through its antioxidant effect.

Besides the oxidative stress, interleukins released during an inflammatory response were found to have a crucial role in pulmonary fibrosis **[1]**. Pulmonary fibrosis is a complex process that involves the release of cytokines, such as TNF-α, IL-6, and monocytes at the site of injury **[30]**. Accordingly, our study explored the inflammatory response mediated by BLM toxicity; we found the levels of TNF-α, NF-κB, and IL-6 in lung tissue to be higher than those of the control group. These findings are in agreement with a previous study, in which the administration of BLM induced high serum concentrations of TNF-α, IL-β1, and NF-κB **[31]**. In addition, pretreatment of rats with chrysin significantly counteracted the increase in the levels of these inflammatory mediators. These findings support the anti-inflammatory effect of chrysin. Multiple studies have mentioned the powerful pharmacological activity of chrysin as an anti-inflammatory substance in different models **[32,33]**.

Following lung injury, oxidative stress and inflammation lead to fibrosis, which is considered a major reason for organ failure **[2]**. The fibroblasts responsible for tissue fibrosis secrete multiple substrates, including latent TGF-β1, extracellular matrix proteins, and collagens, along with ASMA, although not necessarily exist in all injured organs **[34],** particularly in BLM-induced toxicity **[19]**. In our study, greater concentrations of collagen, ASMA, and TGF-β were detected following BLM administration compared to the control group's values. This is consistent with other studies, where BLM administration was responsible for the formation of fibroblastic foci that increase the ability of fibroblasts to colonize the lungs. The study of Tsukui et al. **[35]** revealed that one fibroblast type (Cluster 8 cells) was more effective at inducing migration than alveolar or adventitial fibroblasts. In the present study, pretreatment with chrysin counteracted the effect of BLM on collagen, ASMA, and TGF-β; these findings reflect the antifibrotic effect of chrysin, which is consistent with the studies by **[19, 22, 36]**. Similarly, Li and colleagues **[37]** demonstrated the positive impact that four weeks of chrysin treatment had upon pulmonary tissues. That study also shows chrysin can attenuate pulmonary vascular remodeling and reduce the accumulation of collagen, as well as regulate the expression of collagen I and collagen III.

One important mechanism addressed in the present study is autophagy. This is an intracellular catabolic mechanism associated with infection, inflammation, and cancer **[38]**. Autophagy disruption was reported in IPF (1). The process of autophagy is regulated by multiple autophagy-related (Atg) proteins **[38]**. Autophagic vesicles, particularly cysteine protease ATG4B (Autophagin-1), are accompanied by LC3, which is a protein that mediates the fusion of vesicles with lysosomes **[39]**. A previous study revealed that the

administration of BLM is associated with disruption to the autophagy process **[40]**. Cabrera et al. **[41]** reported that the inflammatory response in ATG4B-deficient mice was heightened at day 7 after BLM administration, presenting as increased neutrophilic infiltration and substantial alterations in pro-inflammatory cytokines. Similarly, Gui and co-workers **[42]** showed that BLM administration was associated with a lower survival rate due to disrupted fibrogenesis. In our study, ATG4B and LC3 were both lower after the administration of BLM. However, administering a 50-mg chrysin pretreatment was effective in counteracting the effect of BLM on ATG4B and LC3; this finding adds a new mechanism for chrysin's protective effect against BLM-induced lung injury. Indeed, recent studies have reported the substantial impact of flavonoid compounds in general on cell proliferation, cancer cell inhibition, and autophagy **[38, 39, 43]**. Lin et al. **[44]** investigated the mechanism of chrysin-induced autophagy in endometrial cancer cells, where the level of LC3II expression was elevated with noticeable enhancement of the autophagic flux, signifying that chrysin influences autophagy and apoptosis. Additionally, chrysin inhibited the phosphorylation of protein kinase B (Akt) and mammalian target of rapamycin (mTOR). This mechanism has led to the recommendation against the use of 5-fluorouracil, which can be replaced by chrysin for the treatment of colorectal cancer.

Furthermore, caspase-1 and the IL33/ST2 signaling pathway were assessed and were found to be significantly higher after BLM administration compared to the control group. IL-33 is an important cytokine released during respiratory diseases, and it is overexpressed in lung diseases such as IPF **[4, 45]**. BLM increased the expression of IL-33 through ST2, yet reduced the full-length form of IL-33 in lung tissue. Exogenous IL-33 is also associated with BLM-induced lung inflammation and fibrosis. Additionally, IL-33 polarizes macrophages to produce IL-13 and TGF-β1 **[45]**. The same conclusion regarding the elevation of IL-33 /ST2 in BLM-treated rats was reached by Xu and colleagues **[46]**. In our study, chrysin was able to mitigate the effect of BLM on the production of Casp-1 and IL33/ST2. Similarly, Nian and colleagues **[47]** have assessed the possible protective effect of chrysin on renal cells after the administration of paracetamol. They found that chrysin reduced the levels of the inflammatory markers, TNF-α, IL-1, and IL-33. Additionally, apoptosis was increased following the increase in Casp-3 activity and LC3B. This result is supported by the findings of another study, where chrysin was used as an anti-cancer therapy in the human breast cancer cell lines, MDA-MB-231 and MCF-7 **[48]**.

Conclusion

The present study provides evidence of the antifibrotic effect of chrysin in a rat model of bleomycin-induced lung fibrosis. Chrysin preserved normal lung function through its effects on the IL33/ST2 signaling pathway, autophagy process, and reduction in oxidative stress and the inflammatory cytokines that are associated with lung fibrosis.

Declarations

Ethics approval and consent to participate

The experimental protocol for the use of animal subjects was approved by the Ethics Committee of the Faculty of Pharmacy of Ain Shams University (license number 78). In addition, the experiments were conducted in accordance with the Animal Research Reporting of In-Vivo Experiment guidelines (ARRIVE guidelines).

Consent to publish

Not applicable

Availability of data and materials

Data will be made available on reasonable request.

Competing interests

The authors declare that no competing interests exist.

Funding Statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author's Contribution

Marwa Nabil: Investigation, Data curation, Formal analysis, Writing - original draft. Haidy E. Michel: Conceptualization, Supervision, review & editing of the manuscript. Esther T. Menze: Conceptualization, Supervision, review & editing of the manuscript. Mohamed F. Abd-Ellah: Supervision and review of the manuscript. Ebtehal El-Demerdash: Conceptualization, Supervision, review & editing of the manuscript.

Acknowledgments:

We thank Dr. Mohamed Abdel-Razek for his assistance in the pathological analysis and Dr. Laila Rashed for biochemical analysis of this work.

5. References

1. Bartold K, Iskierko Z, Sharma PS, Lin HY, Kutner W. Idiopathic pulmonary fibrosis (IPF): Diagnostic routes using novel biomarkers. Biomedical Journal. 2024 Aug 1;47(4):100729.
2. Bezerra FS, Lanzetti M, Nesi RT, Nagato AC, Silva CP, Kennedy-Feitosa E, et al. Oxidative Stress and Inflammation in Acute and Chronic Lung Injuries. Antioxidants (Basel). 2023 Feb 21;12(3):548.
3. Stella GM, D'Agnano V, Piloni D, Saracino L, Lettieri S, Mariani F, et al. The oncogenic landscape of idiopathic pulmonary fibrosis: a narrative review. Translational Lung Cancer Research [Internet]. 2022 Mar [cited 2025 Jan 27];11(3) 472-496. Available from: https://tlcr.amegroups.org/article/view/62151
4. Shakerian L, Kolahdooz H, Garousi M, Keyvani V, Kamal Kheder R, Abdulsattar Faraj T, et al. IL-33/ST2 axis in autoimmune disease. Cytokine. 2022 Oct 1;158:156015.
5. Sun R, Gao DS, Shoush J, Lu B. The IL-1 family in tumorigenesis and antitumor immunity. Seminars in Cancer Biology. 2022 Nov 1;86:280–95.
6. Brandt JP, Gerriets V. Bleomycin. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [cited 2022 Oct 12]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK555895/
7. Mata M, Ruíz A, Cerdá M, Martinez-Losa M, Cortijo J, Santangelo F, et al. Oral N-acetylcysteine reduces bleomycin-induced lung damage and mucin Muc5ac expression in rats. Eur Respir J. 2003 Dec;22(6):900–5.
8. Sogut S, Ozyurt H, Armutcu F, Kart L, Iraz M, Akyol O, et al. Erdosteine prevents bleomycin-induced pulmonary fibrosis in rats. Eur J Pharmacol. 2004 Jun 28;494(2–3):213–20.
9. Rahaman MS, Siraj MA, Islam MA, Shanto PC, Islam O, Islam MA, et al. Crosstalk between xanthine oxidase (XO) inhibiting and cancer chemotherapeutic properties of comestible flavonoids- a comprehensive update. The Journal of Nutritional Biochemistry. 2022 Dec 1;110:109147.
10. Islam MM, Nagaraja S, Hafsa NE, Meravanige G, Asdaq SMB, Anwer MdK. Polyphenol chrysin for management of skin disorders: Current status and future opportunities. Journal of King Saud University - Science. 2022 Jul

1;34(5):102026.

11. Adangale SC, Wairkar S. Potential therapeutic activities and novel delivery systems of chrysin-a nature's boon. Food Bioscience. 2022 Feb 1;45:101316.
12. Mohammed H, Sulaiman G, Albukhaty S, Al-Saffar A, Elshibani F, Ragab E. Chrysin, The Flavonoid Molecule of Antioxidant Interest. ChemistrySelect. 2023 Dec 21;8:1–15.
13. El-Medany A, Hagar HH, Moursi M, At Muhammed R, El-Rakhawy FI, El-Medany G. Attenuation of bleomycin-induced lung fibrosis in rats by mesna. Eur J Pharmacol. 2005 Feb 10;509(1):61–70.
14. Kilic T, Ciftci O, Cetin A, Kahraman H. Preventive Effect of Chrysin on Bleomycin-Induced Lung Fibrosis in Rats. Inflammation. 2014 Dec;37(6):2116–24.
15. Drury RB, Wallington EA, Cameron SE. CARLETON'S HISTOLOGICAL TECHNIQUE. Ulster Med J. 1967;36(2):172.
16. Beutler E. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963;61:882-888.
17. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta. 1978 Nov 15;90(1):37–43.
18. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001 Dec;25(4):402–8.
19. Kseibati MO, Sharawy MH, Salem HA. Chrysin mitigates bleomycin-induced pulmonary fibrosis in rats through regulating inflammation, oxidative stress, and hypoxia. International Immunopharmacology. 2020 Dec;89:107011.
20. El-Khouly D, El-Bakly WM, Awad AS, El-Mesallamy HO, El-Demerdash E. Thymoquinone blocks lung injury and fibrosis by attenuating bleomycin-induced oxidative stress and activation of nuclear factor Kappa-B in rats. Toxicology. 2012 Dec;302(2–3):106–13.
21. Ayilya BL, Balde A, Ramya M, Benjakul S, Kim SK, Nazeer RA. Insights on the mechanism of bleomycin to induce lung injury and associated *in vivo* models: A review. International Immunopharmacology. 2023 Aug 1;121:110493.
22. Deldar Y, Pilehvar-Soltanahmadi Y, Dadashpour M, Montazer Saheb S, Rahmati-Yamchi M, Zarghami N. An *in vitro* examination of the antioxidant, cytoprotective, and anti-inflammatory properties of chrysin-loaded nanofibrous mats for potential wound healing applications. Artificial Cells, Nanomedicine, and Biotechnology. 2018 May 19;46(4):706–16.
23. Huang W, Zhong Y, Gao B, Zheng B, Liu Y. Nrf2-mediated therapeutic effects of dietary flavones in different diseases. Front Pharmacol. 2023 Sep 12 Front. Pharmacol., 2023, 14:1240433 Available from:https://www.frontiersin.org/journals/pharmacology/articles/10.3389/fphar.2023.1240433/full
24. Dai Y, Guo Y, Tang W, Chen D, Xue L, Chen Y, et al. Reactive oxygen species-scavenging nanomaterials for the prevention and treatment of age-related diseases. Journal of Nanobiotechnology. 2024 May 15;22(1):252.

25. Habashy NH, Olleak SA, Abu-Serie MM, Shaban NZ. A new approach for the treatment of bleomycin-induced rat pulmonary injury by the combined protein fraction of major royal jelly protein 2 and its isoform X1. Biomedicine & Pharmacotherapy. 2023 Nov 1;167:115578.

26. Sul OJ, Kim JH, Lee T, Seo KW, Cha HJ, Kwon B, et al. GSPE Protects against Bleomycin-Induced Pulmonary Fibrosis in Mice via Ameliorating Epithelial Apoptosis through Inhibition of Oxidative Stress. Oxid Med Cell Longev. 2022 Mar 20;2022:8200189.

27. Wallach S, Izbicki G, Cohen P, Golan-Gerstl R, Fine A, Breuer R. Bleomycin initiates apoptosis of lung epithelial cells by ROS but not by Fas/FasL pathway. American journal of physiology: Lung cellular and molecular physiology. 2024;290:L790–6.

28. Hanedan B, Ozkaraca M, Kirbas A, Kandemir FM, Aktas MS, Kilic K, et al. Investigation of the effects of hesperidin and chrysin on renal injury induced by colistin in rats. Biomedicine & Pharmacotherapy. 2018 Dec;108:1607–16.

29. Adesina AF, Adewuyi A, Otuechere CA. Exploratory studies on chrysin via antioxidant, antimicrobial, ADMET, PASS, and molecular docking evaluations. Pharmacological Research - Modern Chinese Medicine. 2024 Jun 1;11:100413.

30. Giacomelli C, Piccarducci R, Marchetti L, Romei C, Martini C. Pulmonary fibrosis from molecular mechanisms to therapeutic interventions: lessons from post-COVID-19 patients. Biochem Pharmacol. 2022;193:114812.

31. Ekin S, Yildirim S, Akkoyun MB, Gok HN, Arihan O, Oto G, et al. Theophylline attenuates bleomycin-induced oxidative stress in rats: The role of IL-6, NF-κB, and antioxidant enzymes. Braz J Pharm Sci. 2022;58:e20804.

32. Faheem MA, Akhtar T, Naseem N, Aftab U, Zafar MS, Hussain S, et al. Chrysin Is Immunomodulatory and Anti-Inflammatory against Complete Freund's Adjuvant-Induced Arthritis in a Pre-Clinical Rodent Model. Pharmaceutics. 2023 Apr 12;15(4):1225.

33. Oršolić N, Nemrava J, Jeleč Ž, Kukolj M, Odeh D, Jakopović B, et al. Antioxidative and Anti-Inflammatory Activities of Chrysin and Naringenin in a Drug-Induced Bone Loss Model in Rats. International Journal of Molecular Sciences. 2022 Jan;23(5):2872.

34. Rock JR, Barkauskas CE, Cronce MJ, Xue Y, Harris JR, Liang J, et al. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. Proceedings of the National Academy of Sciences. 2011 Dec 27;108(52):E1475–83.

35. Tsukui T, Sun KH, Wetter JB, Wilson-Kanamori JR, Hazelwood LA, Henderson NC, et al. Collagen-producing lung cell atlas identifies multiple subsets with distinct localization and relevance to fibrosis. Nat Commun. 2020 Dec;11(1):1920.

36. Ahad A, Ganai AA, Mujeeb M, Siddiqui WA. Chrysin, an anti-inflammatory molecule, abrogates renal dysfunction in type 2 diabetic rats. Toxicology and Applied Pharmacology. 2014 Aug;279(1):1–7.

37. Li XW, Wang XM, Li S, Yang JR. Effects of chrysin (5,7-dihydroxyflavone) on vascular remodeling in hypoxia-induced pulmonary hypertension in rats. Chin Med. 2015;10(1):4.

38. Gómez-Virgilio L, Silva-Lucero M del C,

Flores-Morelos DS, Gallardo-Nieto J, Lopez-Toledo G, Abarca-Fernandez AM, et al. Autophagy: A Key Regulator of Homeostasis and Disease: An Overview of Molecular Mechanisms and Modulators. Cells. 2022 Jul 22;11(15):2262.

39. Yang Z, Wilkie-Grantham RP, Yanagi T, Shu CW, Matsuzawa Sichi, Reed JC. ATG4B (Autophagin-1) Phosphorylation Modulates Autophagy. Journal of Biological Chemistry. 2015 Oct;290(44):26549–61.

40. Mahavadi P, Vidyasagar V, Saket A, Shashi C, Lars K, Ingrid H, et al. The pathomechanistic role of autophagy in lung fibrosis. In: Diffuse Parenchymal Lung Disease [Internet]. European Respiratory Society; 2017 [cited 2021 Sep 5]. p. OA478. Available from: http://erj.ersjournals.com/lookup/doi/10.1183/1393003.congress-2017.OA478

41. Cabrera S, Maciel M, Herrera I, Nava T, Vergara F, Gaxiola M, et al. Essential role for the ATG4B protease and autophagy in bleomycin-induced pulmonary fibrosis. Autophagy. 2015 Apr 3;11(4):670–84.

42. Gui YS, Wang L, Tian X, Li X, Ma A, Zhou W, et al. mTOR Overactivation and Compromised Autophagy in the Pathogenesis of Pulmonary Fibrosis. Zhou Y, editor. PLoS ONE. 2015 Sep 18;10(9):e0138625.

43. Pang X, Zhang X, Jiang Y, Su Q, Li Q, Li Z. Autophagy: Mechanisms and Therapeutic Potential of Flavonoids in Cancer. Biomolecules. 2021 Jan 21;11(2):135.

44. Lin YM, Chen CI, Hsiang YP, Hsu YC, Cheng KC, Chien PH, et al. Chrysin Attenuates Cell Viability of Human Colorectal Cancer Cells through Autophagy Induction, Unlike 5-Fluorouracil/Oxaliplatin. Int J Mol Sci. 2018 Jun 14;19(6): E1763.

45. Li D, Guabiraba R, Besnard AG, Komai-Koma M, Jabir MS, Zhang L, et al. IL-33 promotes ST2-dependent lung fibrosis by the induction of alternatively activated macrophages and innate lymphoid cells in mice. Journal of Allergy and Clinical Immunology. 2014 Dec;134(6):1422-1432.e11.

46. Xu J, Zheng J, Song P, Zhou Y, Guan S. IL-33/ST2 pathway in a bleomycin-induced pulmonary fibrosis model. Molecular Medicine Reports. 2016 Aug;14(2):1704–8.

47. Nian JB, Zeng M, Zheng J, Zeng LY, Fu Z, Huang QJ, et al. Epithelial cells expressed IL-33 to promote degranulation of mast cells through inhibition of ST2/PI3K/mTOR-mediated autophagy in allergic rhinitis. Cell Cycle. 2020 May 18;19(10):1132–42.

48. Talebi M, Talebi M, Farkhondeh T, Simal-Gandara J, Kopustinskiene DM, Bernatoniene J, et al. Emerging cellular and molecular mechanisms underlying anticancer indications of chrysin. Cancer Cell Int. 2021 Dec;21(1):214.