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ORIGINAL ARTICLE

Screening of phytochemicals effective on relieving cancer cachexia in cisplatin-induced *in vitro* sarcopenia model

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Abstract

Background Cancer cachexia is a lethal metabolic syndrome induced by cancer and chemotherapy administration and characterized by marked muscle wasting. Although hormone-related cancer therapies have been used for treatment of the cachexia, they have several side effects. Thus, finding out the novel therapeutics for cachexia with minimal side effects is required to allow cancer patients to continue receiving chemotherapies.

Objective In the present study, muscle atrophy was induced by cisplatin on C2C12 myotubes and we examined whether 173 phytochemicals can ameliorate the muscle atrophy in vitro.

Results Cisplatin-induced muscle atrophy upregulated the IL-6 and myostatin expression. Three compounds; magnolol, fisetin, and sclareol markedly inhibited the IL-6 and myostatin expression. They also significantly improved the myotube diameter.

Conclusion Therefore, this study showed the protective effects of three phytochemicals in cisplatin-induced atrophy in vitro and these compounds may be promising therapeutic agents with the further investigation of in vivo potential in the cachexia mice model.

Keywords Cachexia · Cisplatin · Sarcopenia · Muscle atrophy · Phytochemicals

Introduction

Cachexia is defined as a complex metabolic syndrome accompanied with severe clinical outcomes including physical impairment, poor quality of life, poor responses to chemotherapy and poor survival (Orell-Kotikangas et al. 2017). It includes nausea, vomiting, diarrhea, constipation, depression, anxiety, and pain. It is also characterized by weight loss, anorexia and loss of skeletal muscle mass. Cachexia occurs in 15% to 40% of cancer patients and causes the death of about 20% of the patients with advanced cancer or receiving cancer chemotherapies. Patients with cachexia show an increased rate of protein degradation and a decreased rate of protein synthesis in skeletal muscle and as a consequence, the muscle wasting reduces patients' survival and ability to tolerate intensive chemotherapy (Giordano et al. 2003).

The mechanisms of cancer cachexia are still uncertain, but it is known that muscle wasting is related to the proteolysis as a downstream of pro-inflammatory effects including the release of cytokines such as interleukin-1 (IL-1), interferon-γ (INF-γ), IL-6, and tumor necrosis factor-α (TNF-α) (Webster et al. 2020). The pro-inflammatory cytokines can accelerate the muscle wasting by inducing apoptosis or necrosis directly or indirectly. TNF-α is known to be a potent activator of nuclear factor kappa B (NF-κB), permitting NF-κB to translocate to the nucleus in myocytes. This upregulates MAFbx/Atrogin-1 and muscle ring-finger protein-1 (MuRF-1) which are muscle-specific ubiquitin ligases, and myostatin (Mstn) which is a negative regulator of muscle mass in cachexia (Silva et al. 2015). IL-6 also

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plays a pivotal role in muscle injury and repair (Baltgalvis et al. 2008). Increased levels of IL-6 show greater weight loss and poor prognosis of tumors (Kuroda et al. 2007). Thus, the suppression of the production of inflammatory cytokines and the genes related to the proteolysis may be a promising therapeutic approach to cure the cancer cachexia.

In particular, sarcopenia as a pivotal feature of cancer cachexia has been defined as a progressive decline of skeletal muscle mass, strength, and functions in elderly. However, since sarcopenia is rapidly occurred by cancer chemotherapy such as cisplatin, doxorubicin, and etoposide, it is associated with poor clinical outcome, poor quality of life, and increased mortality. In this study, we attempted to develop a new therapeutic agent by performing screening for 173 phytochemicals using an in vitro sarcopenia model.

Cisplatin, a platinum-based anti-cancer drug is widely used as a chemotherapeutic agent for a variety of cancers (Apps et al. 2015). However, cisplatin-based chemotherapy has been reported to be associated with various side effects including nephrotoxicity, ototoxicity, neurotoxicity, and muscle wasting. Cisplatin-induced muscle wasting has been known to be caused by activation of protein degradation and defection of skeletal muscle regeneration. Particularly, cisplatin administration induces a decrease in the expression of MyoD and myogenin, known as major markers of muscle differentiation leading to muscle regeneration. Furthermore, the activation of Mstn signaling by cisplatin induces muscle wasting by activating atrogin-1 and MuRF-1, which are associated with lysosomal proteolysis, through the suppression of Akt signaling and the activation of Smad2 and Smad3 transcription factor complex mediating the inhibition of myogenesis genes (Sakai et al. 2014). Cisplatin chemotherapy has been reported to increase the production of mitochondrial muscle reactive oxygen species (ROS), and the oxidative stress stimulates the production of inflammatory cytokines such as TNF-, IL-1, and IL-6, leading to muscle wasting through activation of p38 and downregulation of Akt (Conte et al. 2020; Jubert Marquez 2020).

Several agents have been used to cure the cachexia, but it is becoming increasingly evident that they may not be completely successful in the treatment of cancer-related muscle wasting. Progestins such as medroxyprogesterone acetate (MPA) and megestrol acetate (MA) are currently used as the best options for cancer cachexia. However, fewer than 30% of patients treated with progestins experience short-term appetite stimulation (Garefalakis et al. 2008), and despite appetite and body weight improvement, there is no evident improvement in survival (Jatoi et al. 2002). In addition, that treatment also has been reported to have side effects such as diabetes, osteoporosis and thromboembolism.

Thus, it is necessary to find out novel therapeutic agents to cure the cachexia with fewer side effects. This study was performed to find out the phytochemicals with the protective potential in cisplatin-induced muscle atrophy in vitro among 173 phytochemical libraries.

Materials and methods

Chemicals

Cisplatin was obtained from Sigma–Aldrich (St. Louis, MO, USA) and resolved in normal saline. 173 phytochemical libraries were obtained from Selleckchem (Houston, TX, USA). The chemicals were resolved in DMSO at 10 mM.

Myotube differentiation

The mouse myoblast cell line C2C12 was obtained from ATCC and maintained in Dulbecco's modified Eagle's medium (DMEM; Welgene, Seongnam, Korea) supplemented with 10% heat-inactivated fetal bovine serum (Welgene), 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen, CA, USA). The cells were seeded in 12-well plates at 1×10^5/well. For differentiation, we switched the medium to DMEM without sodium pyruvate supplemented with 2% heat-inactivated horse serum (GIBCO, Waltham, MA, USA), 100 U/mL penicillin and 100 μg/mL streptomycin. After 5 days of differentiation, the medium was freshly replaced and the myotubes were treated with 10 μM phytochemicals and incubated for 24 h. After 24 h, cisplatin or normal saline was added in each well and incubated for 24 h. Supernatants were collected for ELISA and cells were lysed for mRNA extraction.

Measurement of myotube diameter

Myotube cultures were photographed with an inverted microscope (Olympus, Tokyo, Japan). To estimating the mean value of myotube diameter, the five largest myotubes in five fields for each of the three separate wells per sample were measured by Image J software (NIH, Frederick, MD, USA).

Atrophy scoring

The myotubes were observed under a microscope and captured three different random points using iWorks software (Pixera corporation, Osaka, Japan). The cell damage was graded as following: no damage, –; 0%–30% damage; +, 30%–70% damage; + +, 70%–90% damage; + + +, > 90% damage (cell death); ×.

MTS assay

To estimate cytotoxicity of phytochemicals, C2C12 cells were seeded at 1×10^3 cells/well in 96-well plates and incubated for 5 days. The cells were treated with 10 μM phytochemicals and incubated for 24 h. After 24 h, cisplatin or normal saline was added in each well and incubated for 24 h. 100 μL of MTS solution (Promega, Madison, WI, USA) was added to each well. After 1 h of incubation, the absorbance levels for formazan at 490 and 630 nm were measured using a Microplate Reader (Molecular Devices, Sunnyvale, CA, USA).

Quantitative real-time PCR

Total RNA from cultured C2C12 myotubes were extracted with EasyBlue (Intron, Seongnam, Korea). cDNA synthesis was performed using Cyclescript reverse transcriptase (Bioneer, Daejeon, Korea) following manufacturer's instruction. The expression levels were measured by real-time PCR amplification using SYBR green. Signals are expressed using standard 2-ddCt method after normalizing with the reference signal, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primers were used as following: Myostatin (forward: CAG GCA CTG GTA TTT GGC AG, reverse: TCA GTT ATC ACT TAC CAG CCC AT), GAPDH (forward: ACC CAG AAG ACT GTG GAT GG, reverse: CAC ATT GGG GGT AGG AAC AC).

Cytokine assay

The supernatants cultured from C2C12 myotubes were collected and centrifuged to remove debris. Enzyme-linked immunosorbent assay (ELISA) kits were purchased from BD and Sigma to determine the levels of IL-6. In brief, the samples were loaded in antibody coated 96-well plates and incubated for 2 h at room temperature (RT). The plates were washed and incubated with detection antibodies with streptavidin-HRP for 1 h at RT. TMB solution was incubated for 30 min at RT after washing and the stop solution was added. The absorbance was read at 450 nm.

Western blot

The cells were harvested and lysed in PRO-PREP protein extraction solution (iNtRON, Bio Inc, Sungnam, Korea). Protein concentrations were measured with a Bradford Protein Assay Reagent kit (Bio-Rad, Richmond, CA, USA). Proteins were fractionated by 10% SDS–polyacrylamide gels electrophoresis (PAGE), and transferred onto polyvinylidene difluoride (PVDF) membranes. These were incubated with anti-myostatin and anti-GAPDH Ab (1:1000; Abcam) as primary antibodies. Goat anti-rabbit horseradish peroxidase-conjugated IgG (Abcam, Cambridge, MA, USA) served as secondary antibodies. Protein bands were detected with a chemiluminescence reagent kit (SurModics, MN, USA).

Statistical analysis

Parameters are expressed as mean ± standard error of the mean (SEM). The comparisons were conducted using one-way ANOVA followed by the Newman–Keuls test for multiple comparisons by Prism 5.01 software (GraphPad Software Inc.). $P < 0.05$ was considered to be significant.

Results

Preliminary assessment of the protective effect of phytochemicals

Muscle atrophy and wasting is the critical feature of cachexia. For the screening of phytochemical libraries including 173 compounds, muscle atrophy was induced by 100 μM cisplatin and the compounds were initially tested at 10 μM. The phytochemicals were treated for 24 h and cisplatin was added on phytochemical-pre-treated myotubes. Among 173 compounds, 15 compounds had cytotoxicity and induced cell death. Most of the compounds (over 80 compounds) had no protective effect in cisplatin-induced cell atrophy and showed similar results with cisplatin-treated group accompanied with the severe damage (+++) on the myotubes. The myotubes treated with 43 compounds resulted in 30%–70% damage (++) and those treated with 27 compounds showed light cell damage (+). The screening revealed significant protective potential of the 9 compounds: magnolol, cytisine, artemisinin, hesperetin, fisetin, quercetin dihydrate, catharanthine, sclareol, and licochalcone A, which showed no damage (–). All screening results of myotube observation are shown in Table 1. In addition, cytotoxicity of the phytochemicals was measured by MTS assay, and showed results similar to Table 1 (Supplementary Fig. 1). The representative images of 9 groups are shown in Fig. 1.

Anti-inflammatory activity test of preliminarily screened phytochemicals

To verify whether the inflammatory signal which can be activated by muscle atrophy downstream was reduced by the 9 phytochemicals, IL-6 levels in cell culture supernatants were measured by ELISA. As a result, cytisine, quercetin dehydrate, and catharanthine failed to reduce the IL-6 expression compared to the cisplatin group. Magnolol, artemisinin, hesperetin, fisetin, sclareol, and licochalcone

Table 1 The observation of myotube damages for phytochemical screening

1. Docetaxel	2. Ginkgolide B	3. Lovastatin	4. Andrographolide	5. Baicalin	6. Cryptotanshinone	7. Formononetin	8. Honokiol	9. Luteolin	10. Naringin
x	+	+ + +	+	+ +	+	+ + +	+ +	+ + +	+ + +
11. Oxymatrine	12. Paclitaxel	13. Resveratrol	14. (-)-Epigallocatechin Gallate	15. Apigenin	16. Bergenin	17. Cyclosporin A	18. Ferulic Acid	19. Hyodeoxycholic acid (HDCA)	20. Magnolol
+ +	x	+ + +	+ + +	+	+	+ + +	+ + +	+	-
21. Neohesperidin Dihydrochalcone (Nhdc)	22. Paeonol	23. 2-Methoxyestradiol (2-MeOE2)	24. Reserpine	25. (+)-Usniacin	26. Arbutin	27. Berberine chloride	28. Cytisine	29. Glycyrrhizin (Glycyrrhizic Acid)	30. Icariin
+	+ +	x	+ + +	+ +	+ +	+ + +	-	+ + +	+ +
31. (+)-Matrine	32. Neohesperidin	33. (-)-Parthenolide	34. Vincristine sulfate	35. Hydrocortisone	36. 3-Indolebutyric acid (IBA)	37. Artemether	38. Bilobalide	39. Dihydroartemisinin (DHA)	40. gossypol-Acetic acid
+ +	+ +	+ + +	x	+ + +	+	+ +	+ + +	+ + +	+ + +
41. Indole-3-carbinol	42. Methyl-Hesperidin	43. Nobiletin	44. Phloretin	45. Artemisinin	46. Progesterone	47. 4-Methylumbelliferone (4-MU)	48. Artesunate	49. Caffeic Acid	50. DL-Carnitine HCl
+ + +	+ + +	+ + +	+ + +	-	+	+ + +	+ + +	+ +	+ + +
51. Gramine	52. Kaempferol	53. Morin Hydrate	54. Oleanolic Acid	55. Phlorizin	56. Celastrol	57. Curcumin	58. Esculin	59. Asiatic Acid	60. Chlorogenic Acid
+ + +	+ +	+ + +	+ + +	+ + +	x	+ + +	+ + +	+ +	+ + +
61. Emodin	62. Gynostemma Extract	63. Kinetin	64. Myricetin	65. Oridonin	66. Piperine	67. Costunolide	68. Daidzein	69. Aloe-emodin	70. Azomycin
x	+ + +	+ + +	+ + +	x	+ + +	+ +	+ + +	+ + +	+ + +
71. Chrysin	72. Enoxolone	73. Hesperetin	74. L-(+)-Rhamnose Monohydrate	75. Myricitrin	76. Orotic acid (6-Carboxyuracil)	77. Puerarin	78. Genistein	79. Cyclocytidine HCl	80. Amygdalin
+ + +	+ +	-	+ +	+	+ + +	+ +	+ +	+ + +	+ + +
81. Baicalein	82. Cinchonidine	83. Fisetin	84. Hesperidin	85. Limonin	86. Nalidixic acid	87. Osthole	88. Quercetin Dihydrate	89. Rutaecarpine	90. Sinomenine
+ +	+	-	+ + +	+ +	+ +	+	-	+	+
91. Ursolic Acid	92. Diosmetin	93. Naringin Dihydrochalcone	94. Dihydromyricetin	95. Genipin	96. Guanosine	97. Catharanthine	98. Xylitol	99. Xanthohumol	100. Rutin
+ + +	+ +	+ +	+ +	+ + +	+	-	+ + +	+ + +	+ + +
101. Synephrine	102. Vanillylacetone	103. D-Mannitol	104. Polydatin	105. Sodium Danshensu	106. Geniposidic acid	107. Inosine	108. Schisandrin B (Sch B)	109. Gallic acid	110. Halofuginone

Table 1 (continued)

1. Docetaxel	2. Ginkgolide B	3. Lovastatin	4. Andrographolide	5. Baicalin	6. Cryptotanshinone	7. Formononetin	8. Honokiol	9. Luteolin	10. Naringin
+++	++	++	++	++	++	+++	+	++	x
111. Salicin	112. Tangeretin	113. Xanthone	114. Gastrodin	115. Quercetin	116. Isoliquiritigenin	117. Astragaloside A	118. Forskolin	119. Triptolide (PG490)	120. (+)-Bicuculline
+	++	+	+++	++	+	+++	+++	x	++
121. Cucurbitacin B	122. Sclareol	123. Tanshinone I	124. Yohimbine HCl	125. Hematoxylin	126. Sesamin	127. Sophocarpine	128. Ipriflavone (Osteofix)	129. Scopolamine HBr	130. Hyoscyamine
x	-	+++	++	++	+	+	++	x	+++
131. Piperlongumine	132. Sclareolide	133. Tanshinone IIA	134. 5-hydroxytryptophan(5-HTP)	135. Hordenine	136. Sorbitol	137. Chrysophanic Acid		138. (S)-10-Hydroxycamptothecin	139. 5-Aminolevulinic acid HCl
x	+	+++	+++	+++	+++	+++	x	+++	+++
141. Abscisic Acid (Dormin)	142. Shikimic Acid	143. Taxifolin (Dihydroquercetin)	144. Aloin	145. Indirubin	146. Naringenin	147. Curcumol	148. Apocynin	149. Idebenone	150. Bergapten
+++	+++	+++	+++	+++	+++	+++	+++	+++	++
151. Santacruzamate A (CAY10683)	152. Silibinin	153. Tetrahydropapaverine HCl	154. Biochanin A	155. Lappaconite HBr	156. Salidroside	157. Paeoniflorin	158. Rotundine	159. Vanillin	160. Diacerein
+++	+	++	+++	+	++	+	++	+++	x
161. Licochalcone A	162. L-Glutamine	163. Silymarin	164. Troxerutin	165. Dioscin	166. L-carnitine	167. Palmatine chloride	168. Geniposide	169. Synephrine HCl	170. N6-methyladenosine (m6A)
-	+++	+++	+++	x	++	+	++	+++	+++
171. Calcium D-Pantothenate	172. Oleuropein	173. L-Arginine HCl (L-Arg)			No damage	Damage 0–30	Damage 31–70	Damage 70–90	Dead
+	+++	++			-	+	++	+++	x

The cell damage was graded as following: no damage; −, 0%–30% damage; +, 30%–70% damage; ++, 70%–90% damage; +++, > 90% damage (cell death); ×

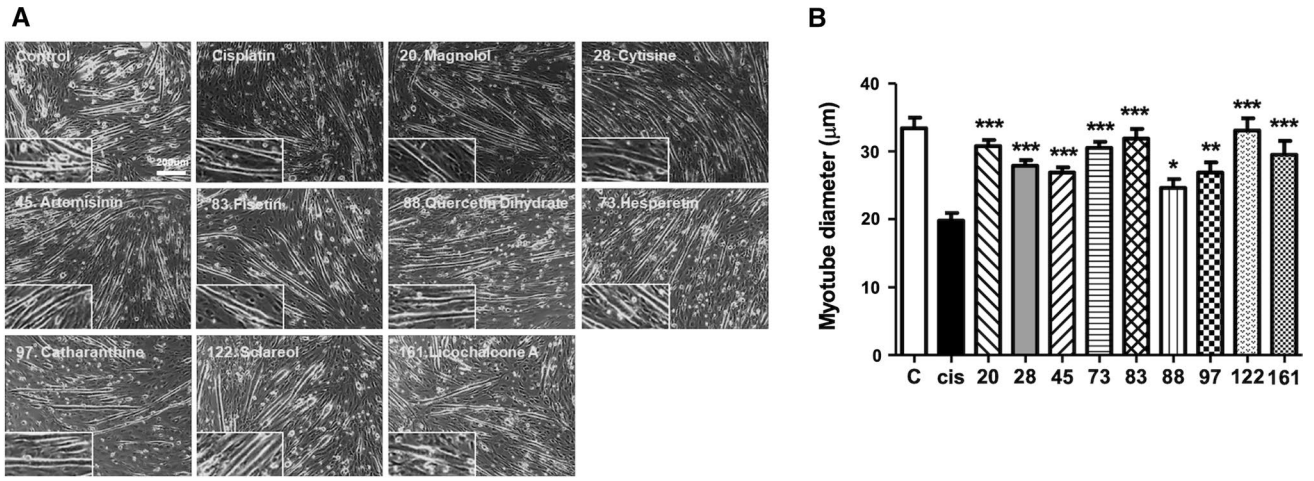

Fig. 1 The protective effects of 9 phytochemicals on cisplatin-induced myotube breakdown. The representative myotube images of 9 phytochemicals (magnolol, cytisine, artemisinin, hesperetin, fisetin, quercetin dihydrate, catharanthine, sclareol and licochalcone **A**) were detected after cisplatin treatment for 24 h. Total magnification, × 10. Scale bar, 200 μm. Inserts show magnified images. (**B**) Quantitative image analysis of myotube diameter. All data are presented as means ± SEMs; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ versus cisplatin (cis)

A showed decreased IL-6 levels compared to the cisplatin group (Fig. 2A).

Test of a proteolytic marker inhibition by preliminarily screened phytochemicals

To clarify the protective effect of 9 phytochemicals, we quantified the mRNA expression of myostatin, which is correlated with muscle atrophy and proteolysis (Zimmers et al. 2002). Similar to the results of IL-6 expression, magnolol, hesperetin, fisetin, sclareol, licochalcone A markedly reduced the myostatin expression compared to the cisplatin group. However, artemisinin failed to lower the myostatin expression. Although cytisine successfully reduced the myostatin level, the IL-6 expression levels did not show any difference between cisplatin and cytisine group (Fig. 2B). Thus, a few compounds, which were not equally effective in reducing IL-6 and myostatin expression, were excluded from the candidates; cytisine, artemisinin, quercetin dihydrate and catharanthine.

Secondary assessment of protective effect of phytochemicals in different concentrations

We further investigated the protective effect of 5 compounds in different concentrations. The observation showed that 0.1 μM to 10 μM magnolol, fisetin, licochalcone A successfully improved the muscle atrophy whereas hesperetin

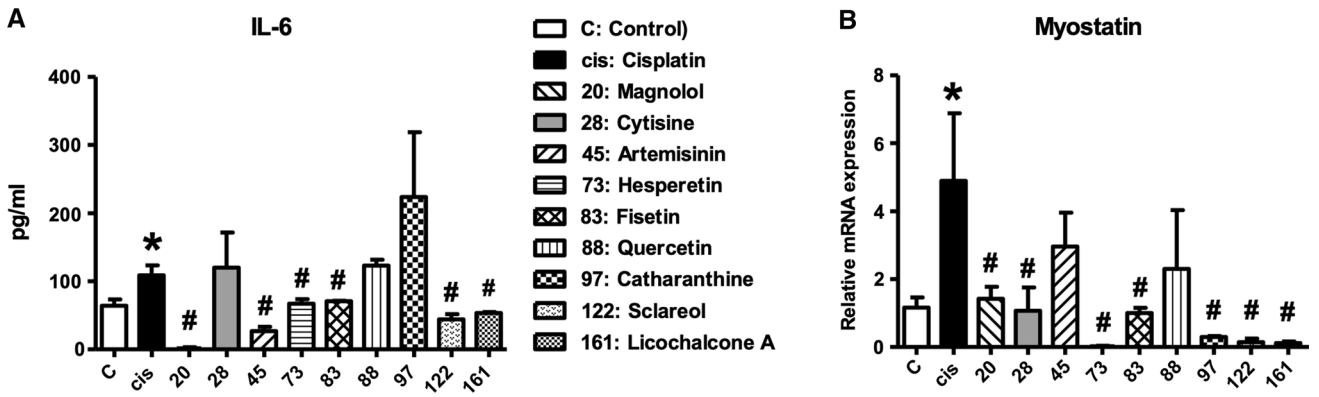

Fig. 2 The effects of 9 phytochemicals on muscle wasting signals. The levels of (**A**) IL-6 and (**B**) myostatin were measured to verify the protective effect of 9 phytochemicals on muscle wasting. The IL-6 expression levels in the cell culture supernatants were detected by ELISA, and the myostatin expression levels were quantified with RT-PCR. All data are presented as means ± SEMs; $*P < 0.05$ versus control (**C**)

showed weak protective effect in all groups. 0.1 μM sclareol failed to protect the myotube, but 1 μM and 10 μM sclareol markedly reduced the muscle wasting. Thus, hesperetin was excluded from the candidates due to the weak protective activity (Fig. 3).

To clarify the observation, IL-6 and myostatin expression levels were measured for 4 compounds excluding hesperetin. Both magnolol and fisetin successfully reduced the IL-6 and myostatin expression. Although 0.1 μM sclareol reduced the IL-6 level but failed to inhibit myostatin expression, 1 μM and 10 μM sclareol markedly reduced the IL-6 and myostatin expression. However, 0.1 μM and 1 μM licochalcone A showed increased IL-6 levels compared to the cisplatin group although the myotube diameter and shape seemed to be similar to the control group. The myostatin levels were almost unchanged in 0.1 μM and 1 μM licochalcone A treatment groups compared to the cisplatin group. Only the 10 μM licochalcone A group showed reduced IL-6 and

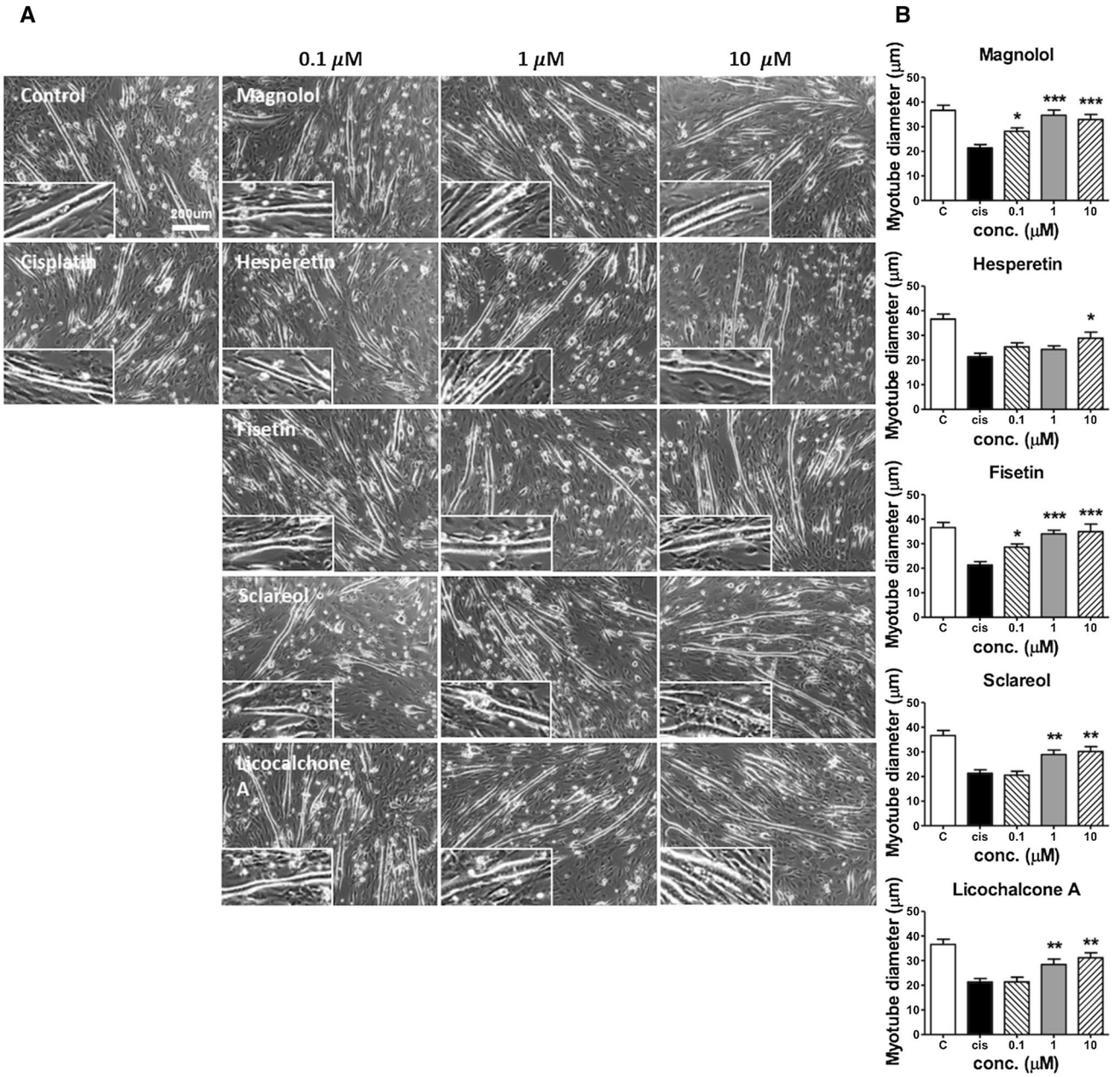

Fig. 3 **A** The protective effects of 5 phytochemicals on muscle wasting in different concentrations. The representative myotube images of control (upper left panel), cisplatin (upper right panel), and 0.1 μM (left), 1 μM (middle), 10 μM (right) phytochemical-treated groups are shown. Total magnification, × 10. Scale bar, 200 μm. Inserts show magnified images. (**B**) Quantitative image analysis of myotube diameter. All data are presented as means ± SEMs; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ versus cisplatin (cis)

myostatin expression (Figs. 4, 5). Additionally, the expression level of myostatin protein was significantly decreased in all groups of magnolol, fisetin, sclareol, and licochalcone A (10 μM) compared to the cisplatin group (Supplementary Fig. 2).

Overall, these results showed that magnolol, fisetin, and sclareol have protective effects in cisplatin-induced cachexia model in vitro.

Discussion

Cisplatin is one of the most common anti-tumor agents which has been clinically used alone or with other anti-cancer drugs including paclitaxel, tegafur–uracil, doxorubicin, etc. as a combination therapy (Dasari et al. 2014). However, it has been reported that cisplatin aggravates the proteolysis and muscle atrophy leading to cachexia.

In this study, we demonstrated that a few phytochemicals effectively alleviated the myotube shrinkage and lowered the atrophic signs such as IL-6 and myostatin. These results revealed that magnolol, fisetin, and sclareol have strong protective effect on cisplatin-induced muscle atrophy in vitro and may be the promising therapeutic agents to cure the cachexia with the further research on in vivo potential in the cachexia mice model.

Magnolol is a multifunctional polyphenolic compound of Magnolia officinalis, possessing anti-oxidative (Chen et al. 2009; Shen et al. 2010), anti-inflammatory (Son et al. 2000), anti-tumor (Ikeda et al. 2002; Li et al. 2007), anti-diabetic (Sohn et al. 2007), and anti-neurodegenerative (Lin et al. 2006) functions. The protective effect of magnolol on cancer cachexia was reported by Chen et al. in 2015 and it was revealed that magnolol suppresses MuRF-1 and MAFbx/atrogin-1 expression in association with inhibition of myostatin and activin A formation and an increase of forkhead box O3 (FoxO3) phosphorylation resulting from

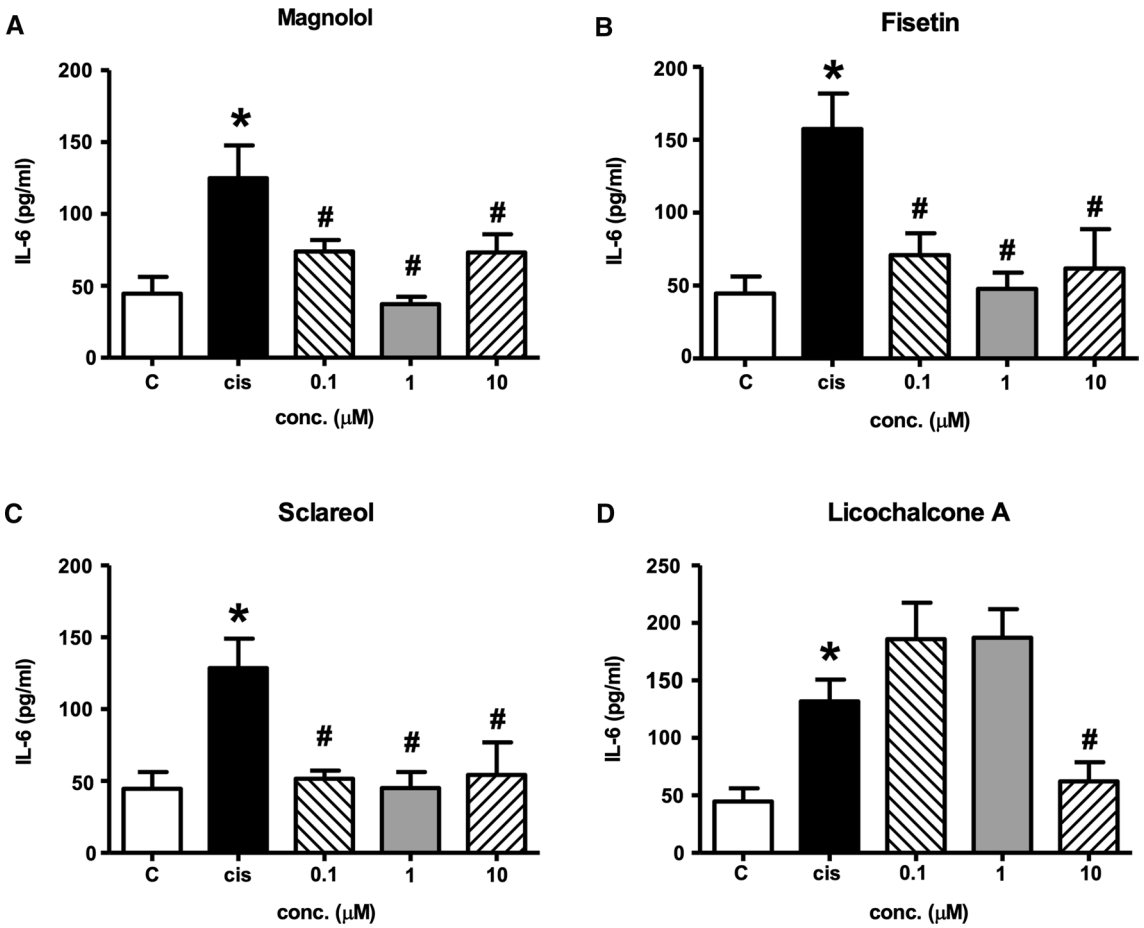

Fig. 4 The changes in IL-6 expression levels in different concentrations by the phytochemical treatment in cisplatin-induced muscle atrophy in vitro. The changes in IL-6 expression levels by (**A**) magnolol, (**B**) fisetin, (**C**) sclareol, and (**D**) licochalcone A treatment in different concentrations were measured by ELISA. All data are presented as means ± SEMs; $*P < 0.05$, $**P < 0.01$ versus control (C), $\#P < 0.05$ versus cisplatin (cis)

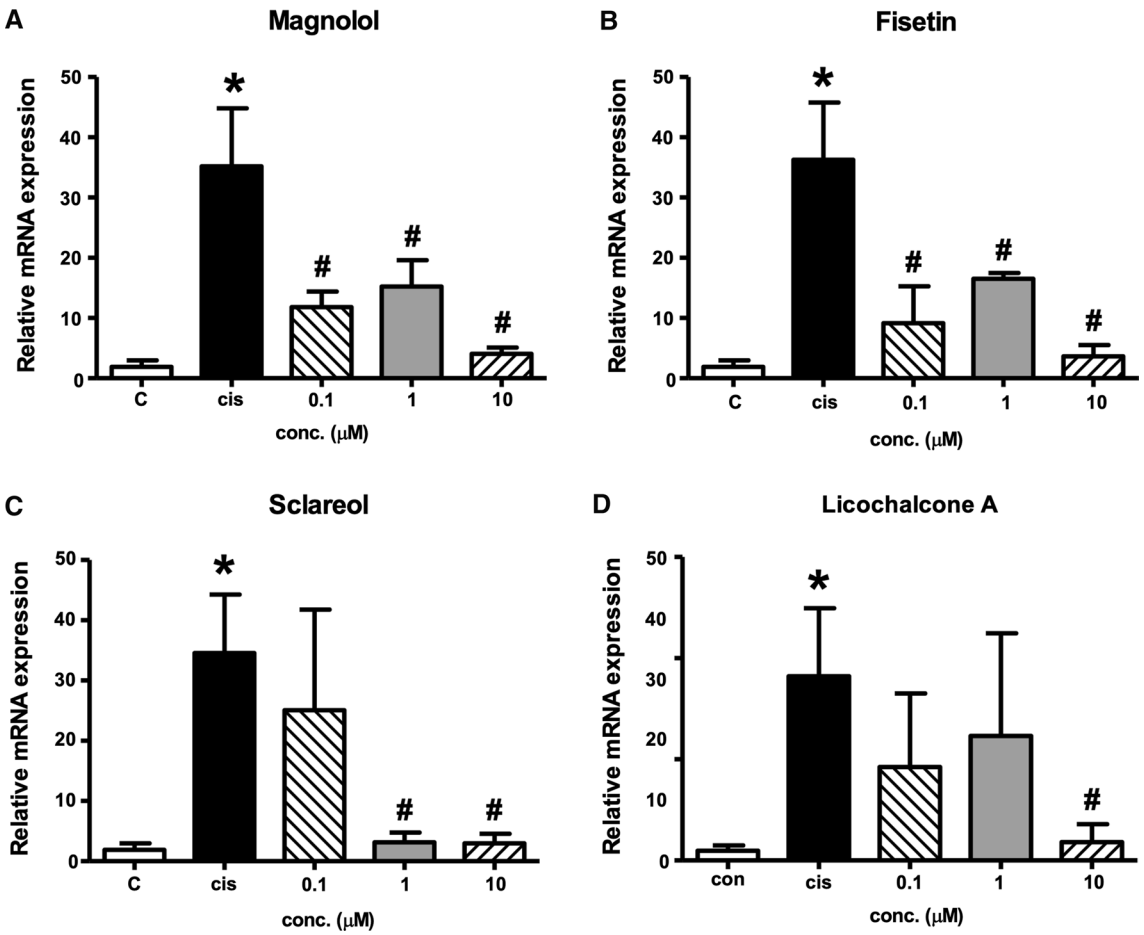

Fig. 5 The changes in myostatin expression levels in different concentrations by the phytochemical treatment in cisplatin-induced atrophy model in vitro. The changes in myostatin expression levels by (**A**) magnolol, (**B**) fisetin, (**C**) sclareol, and (**D**) licochalcone A treatment in different concentrations were quantified by RT-PCR. All data are presented as means ± SEMs; $^{*}P < 0.05$ versus control (C), $\#P < 0.05$ versus cisplatin (cis)

Akt activation (Chen et al. 2015). In our previous study, we reported that magnolol had a therapeutic effect on sarcopenia, a common side effect during cisplatin chemotherapy (Hong et al. 2021; Lee et al. 2020). Therefore, in this study, magnolol also showed potential as a therapeutic agent for sarcopenia by significantly inhibiting IL-6 production and myostatin expression in an in vitro model.

Fisetin is a bioactive flavonol easily found in fruits and vegetables. Sclareol is a bicyclic diterpene alcohol and it is a fragrant compound found in *Salvia sclarea*. It has been reported that both compounds have anti-tumor, antibacterial, antioxidant, and anti-inflammatory activities (Hsieh et al. 2017; Khan et al. 2013). Recently, fisetin was reported to inhibit IL-6/JAK2/STAT3 and TGF-β/Smad3 signaling in the hyperuricemic nephropathy mice (Ren et al. 2021). In addition, sclareol has been reported to exhibit a therapeutic effect by inhibiting the production of inflammatory cytokines such as IL-6, TNF-a, and IL-17 in the rheumatoid arthritis (RA) model. Although they have a variety of bioactivities, the functional mechanisms or the effects on the muscles are still unknown. Thus, further investigation is needed for understanding the action of fisetin and sclareol.

In summary, magnolol, fisetin, and sclareol are the potential agents for muscle atrophy and cachexia.

In conclusion, this study affirms the protective effects of 3 compounds: magnolol, fisetin, and sclareol on cisplatin-induced muscle atrophy in vitro with the protective effect of myotube diameter and the significant decrease in the inflammatory cytokine; IL-6 and the atrophy related gene; myostatin. Thus, we suggest that magnolol, fisetin, and sclareol may be promising as therapeutic agents for cisplatin-induced muscle atrophy. Further investigation is needed to verify the in vivo potential of the compounds in the cachexia mice model.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13273-021-00181-2.

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Declarations

Conflict of interest All author declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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