Effects of Sunphenon and Polyphenon 60 on proteolytic pathways, inflammatory cytokines and myogenic markers in H_2O_2-treated C2C12 cells

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The effect of Sunphenon and Polyphenon 60 in oxidative stress response, myogenic regulatory factors, inflammatory cytokines, apoptotic and proteolytic pathways on H_2O_2-induced myotube atrophy was addressed. Cellular responses of H_2O_2-induced C2C12cells were examined, including mRNA expression of myogenic regulatory factors, such as MyoD and myogenin, inflammatory pathways, such as TNF-α and NF-kB, as well as proteolytic enzymes, such as μ-calpain and m-calpain. The pre-treatment of Sunphenon (50 μg/mL)/Polyphenon 60 (50 μg/mL) on H_2O_2-treated C2C12 cells significantly down-regulated the mRNA expression of myogenin and MyoD when compared to those treated with H_2O_2-induced alone. Additionally, the mRNA expression of μ-calpain and m-calpain were significantly ($p<0.05$) increased in H_2O_2-treated C2C12 cells, whereas pre-treatment with Sunphenon/Polyphenon significantly down-regulated the above genes, namely μ-calpain and m-calpain. Furthermore, the mRNA expression of TNF-α and NF-kB were significantly increased in H_2O_2-treated C2C12 cells, while pre-treatment with Sunphenon (50 μg/mL)/ Polyphenon 60 (50 μg/mL) significantly ($p<0.05$) down-regulated it when compared to the untreated control group. Subsequent analysis of DNA degeneration and caspase activation revealed that Sunphenon (50 μg/mL)/Polyphenon 60 (50 μg/mL) inhibited activation of caspase-3 and showed an inhibitory effect on DNA degradation. From this result, we know that, in stress conditions, μ-calpain may be involved in the muscle atrophy through the suppression of myogenin and MyoD. Moreover, Sunphenon may regulate the skeletal muscle genes/promote skeletal muscle recovery by the up-regulation of myogenin and MyoD and suppression of μ-calpain and inflammatory pathways and may regulate the apoptosis pathways. Our findings suggest that dietary supplementation of Sunphenon might reduce inflammatory events in muscle-associated diseases, such as myotube atrophy.

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1. Introduction

Muscle atrophy occurs as a consequence of numerous pathological conditions, including cachexia and disuse-related events due to oxidative stress (Smith *et al.* 2008). The disuse-induced muscle atrophy results from both increased proteolysis and decreased protein synthesis (Wing 2005). Oxidative stress is considered a key regulator of cell signaling pathways leading to increased proteolysis and muscle atrophy during periods of prolonged disuse (Powers *et al.* 2007). Hydrogen peroxide, which causes oxidative stress, can contribute to disused muscle atrophy by reducing myogenic differentiation markers (Myogenin and MyoD) and also by increasing proteolysis (μ-calpain, m-calpain) in skeletal muscles (Prochniewicz *et al.* 2008; Murray *et al.* 2013) and myotubes (Li *et al.* 2005).

Activation of μ-calpain, m-calpain and caspase-3 has been observed in muscle atrophy, and is responsible for

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releasing the myofilament during the initial stages of muscle atrophy (Powers *et al.* 2007). Moreover, inflammatory markers (TNF-α and NF-kB) and caspase-3 can also affect satellite cell activation and differentiation, which could lead to skeletal muscle atrophy (Degens 2010). Cultured C2C12 muscle cells provide a fast and reliable system for studying muscle differentiation especially myogenesis. Some growth factors, viz. cytokines and oncogenes, repress the activity of myogenin and inhibit myogenesis, thus resulting in decreased muscle mass i.e. muscle atrophy (Wust and Degens 2007). Myogenesis is often associated with chronic inflammation, because the tumour necrosis factor (TNF-α) and nuclear factor-kB (NF-kB) have been implicated as major mediators of cachexia (muscle atrophy). Cell–cell communications have significant influence on cell density and play an important role in myogenic differentiation (myogenesis). Muscle inflammation has been shown to initiate the degradation of intrinsic muscle proteins and to inhibit myogenesis by suppression of differentiation formation in C2C12 cells (Kislinger *et al.* 2005). Activated TNF-α and NF-kB are sufficient to block myogenesis, which interferes with the expression of muscle proteins in differentiating myoblasts. Conversely, activation of NF-kB by over-expression of IkB kinase was sufficient to block myogenesis, illustrating the causal link between NF-kB activation and inhibition of myogenic differentiation.

Remarkably, in many conditions, muscle atrophy is associated with chronic elevations in circulating inflammatory cytokines, in particular TNF-α, resulting in disturbance of energy or anabolism catabolism balance (Langen *et al.* 2001). TNF-α and NF-kB were believed to trigger the events leading to muscle wasting (Muscle atrophy). In support of this, chronic administration of TNF-α or IL-1 induced weight loss and skeletal muscle wasting in rats (Ruscin *et al.* 2005). Muscle wasting may alternatively result from a decrease of the number of fibers.

Green tea is made from the dried leaves of *Camellia sinensis,* a perennial evergreen shrub. Green tea, black tea and oolong tea are all derived from the same plant. They are produced and consumed primarily in China, Japan, and countries in North Africa and the Middle East. Green tea retains higher levels of catechins, which are highly antioxidant polyphenolic compounds. Many of the potential cancer-preventive effects of green tea are thought to be mediated by its most abundant Sunphenon-/Polyphenon-containing catechin, epigallocatechingallate (EGCG) (Maeta *et al.* 2007). Green tea is a source of caffeine: one cup of tea contains approximately 50 mg of caffeine, depending on the strength and size of the cup (as compared to coffee, which contains 65–175 mg of caffeine per cup). In our study, we analysed the effect of green tea polyphenons, such as Sunphenon and Polyphenon 60, on expression of mygenesis (Myogenin and MyoD), inflammatory cytokines (TNF-α and NF-kB), proteolytic enzymes (μ-calpain and m-calpain) and DNA fragmentation on H_2O_2-induced apoptotic condition in C2C12 cells.

2. Materials and methods

2.1 *Chemicals*

The mouse myoblast C2C12 cells were purchased from American Type Culture Collection (ATCC CRL-1772). The iScriptTM cDNA synthesis kit (170-8891) and SsoFast Eva-Green Supermix (172-5202) were purchased from BIO-RAD (BIO-RAD Laboratories, Inc., USA). Primers were purchased from Genotech (Daejeon, South Koea). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco®Life Technologies, Grand Island, NY, USA. Sunphenon, Polyphenon-60 and cell growth determination kit were purchased from Sigma-Aldrich. All laboratory wares were purchased from Falcon Labware (Becton-Dickinson, Franklin Lakes, NJ, USA).

2.2 *Cell culture*

The C2C12 cells were grown in 75 cm^2 flasks incubated at a density of 7,000 cells/cm^2 and grown in DMEM supplemented with 10% FBS and penicillin (100 U mL^{-1}) in humidified atmosphere with 5% CO_2, at 37°C. After reaching 80–90 % confluence, cells were harvested from subconfluent monolayers after their detachment by exposure to trypsin containing 5.3 mM EDTA for 5 min at 37°C. Cells were then shifted into differentiation medium (10% FBS replaced with 2% Horse Serum) for differentiation of the cells to myotubes. After 3 to 5 days of culture, a large proportion of long multi-nucleated myotubes were observed among the myoblasts. These cells were utilized for the further studies. Cell culture studies demonstrate that multinucleated myotubes form in a series of ordered steps. Initially, myoblasts differentiate into elongated myocytes that migrate, adhere and fuse to one another to form small nascent myotubes that contain few myonuclei. Nascent myotubes further fuse with additional myocytes or with other myotubes to generate mature myotubes that contain many myonuclei.

2.3 *Experimental groups*

C2C12 cells were treated with polyphenon (50 μg/mL) and/or sunphenon (50 μg/mL) for 24 h and then treated with H_2O_2 (20 μM, 4 h). After incubation for 4 h, different cellular and molecular end points were studied.

Group 1: control cells
Group 2: control+polyphenon (50 µg/mL) treated
Group 3: control+sunphenon (50 µg/mL) treated
Group 4: control+H_2O_2 (20 µM, 4 h) treated
Group 5: control+polyphenon (50 µg/mL) + H_2O_2 (20 µM, 4 h)
Group 6: control+sunphenon (50 µg/mL) + H_2O_2 (20 µM, 4 h) treated

After treatment, the cells were used for further studies.

2.4 *Cell viability assay*

Cultured C2C12 cells at a concentration of 1×10^6 cells/mL were taken in a 96-well plate. Subsequently, the cells were pretreated with Sunphenon (50 µg/mL) and Polyphenon (50 µg/mL) for 24 h, followed by the addition of 20 µM $H_2O_2$and incubated for 4 h. Then, MTT solution, in an amount equal to 10% of the culture volume, was added under sterile conditions and incubated for 3 to 4 h. After the incubation period, the cultures were removed from the incubator and the resulting MTT formazanwas dissolved. Gentle stirring using a gyratory shaker enhanced the dissolution. Then, MTT solvent, in an amount equal to the original culture volume, was directly added to the culture and the plates were read within 1 h at 570 nm using an ELISA- type plate reader equipped with appropriate filters.

2.5 *Apoptotic morphology (nuclear condensation) by acridine orange–ethidium bromide dual staining*

Acridine Orange (AO) and Ethidium Bromide (EtBr) staining are used to detect the condensed chromatin of dead apoptotic cells (Reddy and Prasad 2011). Apoptotic cells uptake EtBr and emits red/orange fluorescence under 550 nm. Acridine orange is a DNA-selective and membrane-permeable fluorescent cationic dye that freely enters normal cell nuclei and emits green fluorescence under 525 nm. Stained cells were viewed under fluorescence microscope (Carl Zeiss, Jena, Germany). During apoptosis, DNA becomes condensed and fragmented. The apoptotic cells were counted as a function of the total number of cells present in the field.

2.6 *Isolation of RNA and quantitative real time polymerase chain reaction (qRT-PCR)*

After treatment, Total cellular RNA was extracted with the RNeasy^{TM} mini kit in accordance with the manufacturer's instructions from QIAGEN, RNeasy Mini Kit (Cat# 74104 and 74106). cDNA was synthesized using 2 µg total RNA by iScript^{TM}cDNA Synthesis Kit from Bio-Rad. Real-time PCR was performed using a cDNA equivalent of 10 ng of the total RNA from each sample with primers specific for Myogenin, MyoD, TNF-α, NF-κB, µ-calpain, m-calpain, caspase-3, Hsp27, Hsp70, and a housekeeping gene, *GAPDH* (table 1). The reaction was carried out in 10 µL using SsoFast™ EvaGreen® Supermix (Bio-Rad, Hercules, CA) according to the manufacturer's instructions.The RT-PCR assays were performed using CFX96™ Real-Time PCR detection system (Bio-Rad).The statistical analysis of the real time PCR results was calculated by using the ΔCt value (Ct gene of interest – Ct reporter gene). Relative gene expression was obtained by ΔΔCt methods (ΔCt sample - ΔCt calibrator), with the use of the sham-operated group as a calibrator for comparison of all unknown sample gene expression levels. The conversion between ΔΔCt and relative gene expression levels is as follows: fold induction = 2-ΔΔCt, where 2-ΔΔCt is relative gene expression (Livak and Schmittgen 2001).

2.7 *Statistical analysis*

The data on the biochemical analysis of the present study represent mean values±SD. Statistical evaluation was carried out by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). A value of $p<0.05$ was considered statistically significant.

3. Results

We assessed the effect of Sunphenon and Polyphenon-60 on H_2O_2-induced C2C12 cell viability using MTT assays. Figure 1 shows that the cytotoxicity was greatly increased ($p<0.05$) in H_2O_2-induced cells. Cell death was significantly decreased upon pre-treatment with Sunphenon and Polyphenon in H_2O_2-induced C2C12 cells. Figure 2(i) show the effects of Sunphenon and Polyphenon on apoptotic morphological changes of all groups. Arrows shows 75% of apoptotic cells in H_2O_2-treated C2C12 cells (D), pretreatment of SP (E) and PP (F) in H_2O_2-induced cells decreased apoptotic cells 16% and 22% respectively, compared to H_2O_2-induced alone. The percentage of apoptotic cell is shown in figure 2(ii).

We analysed the mRNA levels of Myogenin and MyoD related to myogenic differentiation markers (table 1). The mRNA expression of Myogenin and MyoD were significantly ($p<0.05$) down-regulated in H_2O_2-induced cells when compared to control cells. Upon prior treatment with Sunphenon and Polyphenon in H_2O_2-treated cells, the expression of Myogenin and MyoD were up-regulated when compared to H_2O_2-alone-treated cells. The mRNA levels were similar between CON, CON + PP and CON + SP groups. We next measured the mRNA expressions of the proteolytic-related

Table 1. Effects of SP and PP 60 on proteolytic, inflammatory and myogenic markers of gene expression analysed by qRT-PCR in H_2O_2-treated C2C12 cells

Gene	CON	CON+PP 60	CON+SP	H_2O_2	PP 60+H_2O_2	SP+H_2O_2
Myogenin	1.0 ± 0.06	1.1 ± 0.06	1.15 ± 0.1	0.4 ± 0.03[a]	0.9 ± 0.05[b]	0.85 ± 0.05[b]
Myo D	1.0 ± 0.05	1.18 ± 0.09	1.05 ± 0.05	0.35 ± 0.02[a]	0.86 ± 0.04[b]	0.97 ± 0.05[b]
μ-calpain	1.0 ± 0.05	0.9 ± 0.04	1.09 ± 0.05	1.80 ± 0.09[a]	1.21 ± 0.06[b]	1.23 ± 0.06[b]
m-calpain	1.0 ± 0.05	0.99 ± 0.04	1.12 ± 0.06	1.41 ± 0.07[a]	1.15 ± 0.06[b]	1.12 ± 0.05[b]
TNF-α	1.0 ± 0.07	1.02 ± 0.07	1.05 ± 0.07	4.80 ± 0.3[a]	1.75 ± 0.12[b]	1.98 ± 0.13[b]
NF-kB	1.0 ± 0.06	0.98 ± 0.06	1.02 ± 0.06	6.8 ± 0.4[a]	1.84 ± 0.11[b]	1.88 ± 0.11[b]
Cas-3	1.0 ± 0.08	1.03 ± 0.08	1.02 ± 0.08	3.2 ± 0.2[a]	1.84 ± 0.14[b]	1.88 ± 0.15[b]
Hsp-27	1.0 ± 0.05	0.93 ± 0.04	1.0 ± 0.05	1.53 ± 0.08[a]	1.15 ± 0.05[b]	1.12 ± 0.06[b]
Hsp-70	1.0 ± 0.05	1.08 ± 0.05	0.96 ± 0.04	1.87 ± 0.09[a]	1.32 ± 0.07[b]	1.23 ± 0.06[b]

The mRNA expressions of specific genes were quantified by qRT-PCR.Values are means ± SD from each group. CON – control cells; SP – Sunphenon treated cells; PP – Polyphenon treated cells; SP+H_2O_2 - H_2O_2 cells pre-treated with SP (50 μg); PP+H_2O_2 – H_2O_2 cells pre-treated with PP (50 μg); CON + SP - control cells treated with SP (50 μg); CON + PP – control cells treated with PP (50 μg). [a] Significant as compared with control cells (p<0.05) (DMRT); [b] significant as compared with H_2O_2 cells (p<0.05) (DMRT).

genes such as μ-calpain and m-calpain.The H_2O_2-treated cells showed significantly (p<0.05) increased expression of μ-calpain and m-calpain when compared to the control cells. The pre-reatment with Sunphenon and Polyphenon decreased the μ-calpain and m-calpain mRNA expression in H_2O_2-treated C2C12 cells. The Sunphenon and Polyphenon treated cells did not show any significant effect compared to control cells. We also measured the effects of Sunphenon (SP) and Polyphenon (PP) on H_2O_2-induced inflammatory cytokines production such as TNF-α and NF-kB from C2C12 cells. The results of these genes were significantly (p<0.05) up-regulated in the H_2O_2-treated cells, and at the same time the pre-treatment with Sunphenon and Polyphenon down-regulated the mRNA expression of TNF-α and NF-kB in H_2O_2-induced myotubes. Finally, we observed the mRNA expressions of caspase-3, Hsp-27 and Hsp-70 in all six groups: these mRNA expressions were significantly (p<0.05) increased in H_2O_2 induced cells. whereas they were decreased in Sunphenon and Polyphenon pretreated groups in H_2O_2-induced cells. The numeric values of above all mRNA expression of these specific genes are shown in table 1.

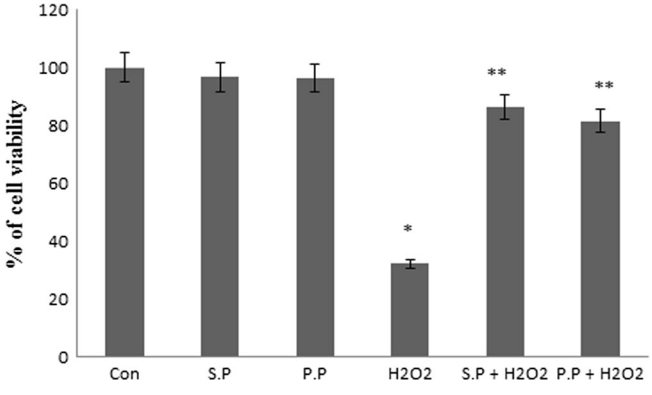

Figure 1. S.P and P.P inhibits cell death in H_2O_2 induced C2C12 cells. C2C12 cells were treated with 20 μM H_2O_2 for 4 h and cell proliferation was detected by MTT assay. *Significant as compared to control; **Significant as compared to H_2O_2-treated cells. Values are mean±SD of three independent determinations. (p<0.05, ANOVA followed by DMRT).

4. Discussion

Oxidative stress is frequently associated with chronic inflammation and it could cause endothelial dysfunction as well as cellular injury, which in turn leads to muscular atrophy (Lei *et al.* 2014; Vester *et al.* 2014). Hence, we investigated the effect of Sunphenon and Polyphenon 60 on H_2O_2-induced myotube atrophy with a view to elucidate the potential protective mechanisms. The results of the study suggest that Sunphenon, a water-soluble polyphenol-rich fraction of green tea extract, acts as an antioxidant by scavenging the free radicals. First, these results demonstrate that low levels of H_2O_2 are sufficient to induce myotube oxidative stress and atrophy in C2C12 myotubes within 4 h and is characterized by decreases in the mRNA expression of Myogenin and MyoD (table 1). As far as the metabolism of skeletal muscle cells are concerned, oxidative stress affects the expression of the redox-sensitive genes involved in protein synthesis (Yang *et al.* 2014) and inhibits the myogenesis at the level of muscle-specific protein expression (Willkomm *et al.* 2014). Pro-oxidant compounds are increased in muscle cell under oxidative stress and reduced-to-oxidized glutathione ratio, which suggests the impairment of myogenic regulatory factors such as Myogenin and MyoD. It has been demonstrated that free radicals affect the expression of redox-sensitive genes involved in myogenic differentiation and H_2O_2 inhibits myogenesis in cell culture (Wang *et al.*

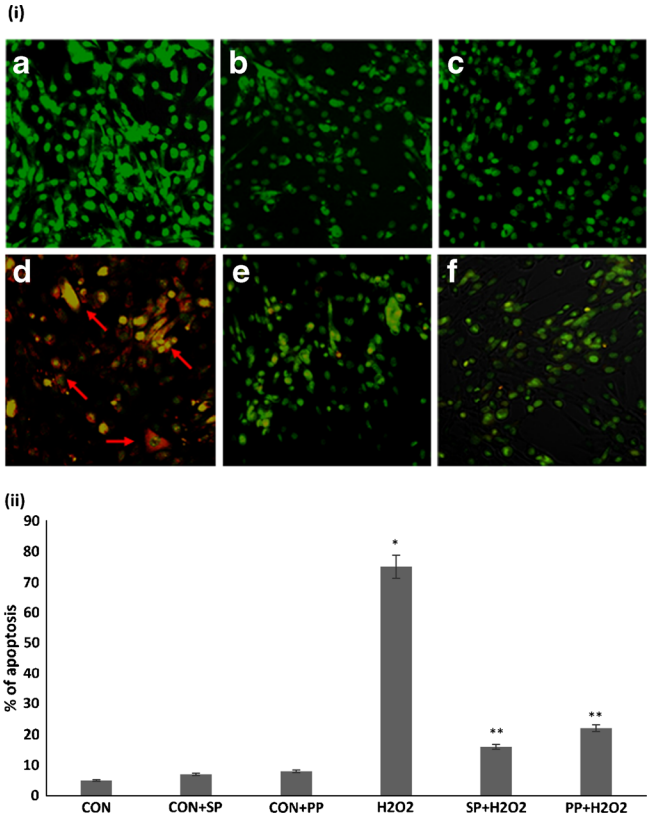

Figure 2. (**i**) Cellular morphological changes were observed under a fluorescence microscope using AO/EtBr staining (×20). The normal appearing morphology were observed in control (a), SP (b) and PP (c) treated alone cells. More number(75 %)of apoptotic cells (arrows on red/ orange colour) were observed in H_2O_2-treated C2C12 cells (d) when compared to control (a); the pretreatment of SP (50 µg/mL) (e) and PP (50 µg/mL) (f) were decreased the frequency of H_2O_2- induced apoptotic cells. (**ii**) Bar graph represents the quantitative comparison between the groups. Values are given as means±SD of six experiments in each group. *Significant as compared to control; **Significant as compared to H_2O_2 treated cells.

2014). Second, µ-calpain and Ca^{2+} are involved in the H_2O_2-induced atrophy, and this increased the µ-calpain activation and can cause the muscle atrophy due to Ca^{2+} influx (table 1), which suggested that the activation of the µ-calpain is a required for H_2O_2-induced atrophy (McClung *et al.* 2009).

In the same way, the muscle-derived oxidants and inflammatory cytokines, namely TNF-α, NF-kB and IL-6, contribute to skeletal muscle repair via the regulation of caspase-dependent apoptosis (caspase-3 and caspase-7) and the activation of proteolysis (µ-calpain and m-calpain) (Andrianjafiniony *et al.* 2010). We and others have hypothesized that disturbances in redox balance are critical regulatory mechanisms for the activation of both the Ca^{2+}-dependent µ-calpain, m-calpain and caspase-3 during atrophy and that these proteases are responsible for myofilament release during the initial stages of muscle atrophy (Servais *et al.* 2007). H_2O_2-induced atrophy and cellular oxidative stress result in the activation of calpain, caspase, TNF-α and HSPs lead to myotube atrophy; a direct link between the calcium release, oxidative stress, inflammation and apoptosis has been definitively proven in the current study (figure 3). Results from our study show that pre-treatment with Sunphenon/ Polyphenon 60 to H_2O_2 delivery effectively prevent the formation of muscle atrophy by mediating the proteolysis, apoptosis and inflammatory pathways. These results suggest that Sunphenon and Polyphenon 60 played a critical role in inhibiting apoptosis through their protective action on DNA (figure 2(i)),which might be due to their free radical scavenging abilities and phenolic constituents (Kaviarasan *et al.* 2011).

Moreover, the activation of inflammatory markers affect satellite cell activity (proliferation and differentiation) by the suppression or breakdown of Myogenin and MyoD, which are the key regulators of the transition from proliferation to differentiation of the satellite cell (Degens 2010). H_2O_2 is reported to induce the production of inflammatory cytokines such as TNF-α, TGF-β and NF-kB that could result in muscle atrophy, both *in vitro* and *in vivo* (Lee and Choi 2005). Among these cytokines, TNF-α exerts a key role in the cytokine network with regard to the pathogenesis of many infectious and inflammatory diseases (Bertazza and Mocellin 2008). TNF-α induces a variety of cellular responses, including cell survival, cell proliferation, paradoxically and cell death leading to apoptosis by the activation of caspase-3 and caspase-7 (Varfolomeev and Ashkenazi 2004; Wang *et al.* 2008). Apoptosis is an important biological event consisting of cell death in many biological systems and it could induce inflammatory response (Berridge 2012). In the present study, it was demonstrated that H_2O_2 induces TNF-α production in C2C12 cells, which in turn activates apoptotic pathways because of TNF-α which may activate the caspase-3 and NF-kB (table 1 and figure 3), Bax and release of cytochrome c, thereby providing a link between the apoptosis and inflammation in H_2O_2-induced apoptosis (Deng *et al.* 2003). Similarly, chronic elevation in TNF-α may contribute to elevated TGF-β1 mRNA expression in apoptosis.

The increase in TGF-β1 gene expression during TGF-β1 stimulates the growth of myoblasts (Sullivan *et al.* 2009). Moreover, caspase-3 rapidly induces apoptosis, by NF-κB activation (Hu *et al.* 2000). Therefore, we concluded interlinking the inflammation and apoptosis pathways, and these data suggested that caspase-3 may activate NF-kB (table 1). These apoptotic pathways are regulated by interactions with heat shock proteins (HSPs), which play a critical role in cell stress to prevent the appearance of folding intermediates that lead to misfolded or otherwise damaged molecules (Jolly and Morimot 2000; Mayer and Bukau

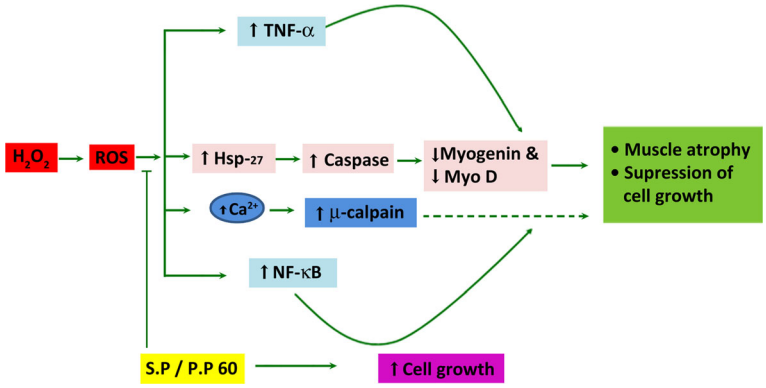

Figure 3. Schematic representation of the major molecular sequential steps of muscular atrophy or myotube atrophy induced by H_2O_2. H_2O_2 generates oxidative stress leads to muscular atrophy and suppression of cell growth through ROS by three main pathways: 1. Activation of TNF-α which may activate NF-κB. 2. Activation of Hsp-27 which mayactivate Caspases. 3. Activation of μ-calpain through Ca^{2+} channel. ⊣ indicates inhibition.

2005). We obtained an increase in expression of HSP-70 (table 1) after exposure to hydrogen peroxide induction, suggesting that HSP-70 acts as sensor and regulator of stress-induced apoptosis (Singh *et al.* 2007). In fact, antioxidant administration attenuates myofiber atrophy in both disease and disuse models (Minnaard *et al.* 2006; Servais *et al.* 2007). Our findings of the study suggested that Sunphenon and Polyphenon both have stronger antioxidant, anti-inflammatory and anti-apoptotic activity, and thus might reduce myotube atrophy. These findings are consistent with that of Ota *et al.* (2011), who reported improvement in soleus muscle mass in mice that were fed catechins during hind-limb unloading. Patients with increased oxidative stress levels suffering from chronic diseases could benefit from Sunphenon and Polyphenon 60 supplementation.

5. Conclusion

In conclusion, H_2O_2, a free radical generator, significantly induced cell death and improve the inflammation by the activation of TNF-α and NF-kB in C2C12 cells. The activation of TNF-α could induce apoptosis via caspase-3 activation and up-regulate HSP-27 and HSP-70. Pre-treatment with Sunphenon and Polyphenon inhibited this process by suppressing the inflammatory pathways by down-regulating TNF-α and NF-kB as well as apoptosis pathways through the suppression of caspase-3 and DNA fragmentation in H_2O_2-induced C2C12 cells. On the other hand, Sunphenon and Polyphenon treatment enhanced the activity of Myogenin and MyoD in muscle atrophy. These inferences suggest that Sunphenon and Polyphenon 60 promote cell growth and cell survival against H_2O_2-induced C2C12 myoblast by the activation of myogenic program (i.e. increased Myogenin and MyoD expression), and suppression of inflammatory pathways (i.e. TNF-α and NF-kB). Hence, application of Sunphenon and Polyphenon 60 appears to be a possible new therapeutic approach towards treatment for inflammation-induced muscle atrophy or prevention of oxidative stress.

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