PROTECTIVE EFFECTS OF SALIDROSIDE AND TRICHOSTATIN A AGAINST SIMVASTATIN-INDUCED SKELETAL MYOPATHY: HISTOPATHOLOGICAL EVALUATION AND C2C12 CELL-LINE INVESTIGATION

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Abstract: **Background**: Statins are widely prescribed lipid-lowering agents, but their use can be limited by skeletal muscle toxicity ranging from myalgia to severe myopathy. Oxidative stress, mitochondrial dysfunction, apoptosis, and impaired autophagy are implicated in statin-associated muscle injury. Salidroside (a Rhodiola rosea phenolic glycoside) shows antioxidant and mitochondrial protective activity, while Trichostatin A (TSA), a histone deacetylase inhibitor, may modulate autophagy and apoptosis—suggesting potential synergy as an adjunct strategy. **Objective**: To evaluate the protective potential of Salidroside and TSA (alone and in combination) against simvastatin-induced myopathy using (i) in vivo functional and biochemical assessment with histology, and (ii) in vitro C2C12 cell-line assays. **Methods**: Male Wistar rats (200–250 g) were randomized into treatment groups including simvastatin (80 mg/kg p.o.) and adjunct Salidroside (50 mg/kg i.p.) and/or TSA (0.6 mg/kg i.p.) for 15 days. Functional tests (rota-rod, open-field, righting reflex), serum CK/LDH, and H&E-based muscle histology grading were planned. In vitro, C2C12 myoblast viability was assessed by trypan blue counting, morphology, and MTT assay. **Expected outcomes:** Simvastatin is expected to elevate CK/LDH and worsen motor performance with degenerative histological changes; adjunct Salidroside and TSA are expected to reduce biochemical injury markers and improve histological and cell-viability outcomes. **Conclusion**: This integrated histology + cell-line design provides a translational framework to test Salidroside and TSA as potential adjunct candidates to mitigate statin-associated myopathy. **Keywords**: Statin-induced myopathy; Simvastatin; Salidroside; Trichostatin A; HDAC inhibitor; C2C12; Histopathology; Creatine kinase; Lactate dehydrogenase

1. Introduction

Statins are the most widely prescribed lipid-lowering agents and represent the cornerstone of cardiovascular disease prevention (Grundy et al., 2019). Despite their proven clinical benefits, long-term statin therapy is frequently associated with skeletal muscle adverse effects collectively termed statin-associated muscle symptoms (SAMS) (Stroes et al., 2015). These manifestations range from mild myalgia to severe myopathy and rhabdomyolysis, often leading to poor patient compliance and discontinuation of therapy Thompson et al., 2016).

Simvastatin, a lipophilic HMG-CoA reductase inhibitor, has been reported to induce muscle toxicity more frequently than hydrophilic statins, particularly at higher doses (Vinci et al., 2021). The pathogenesis of statin-induced myopathy is multifactorial and involves mitochondrial dysfunction, oxidative stress, impaired autophagy, apoptosis, and structural damage to myofibers (Bouitbir et al., 2020). Histologically, statin-induced myopathy is characterized by myofiber vacuolization, hyalinization, fiber splitting, centrally located nuclei, and widening of interstitial spaces (Goodman et al., 2015).

Currently, there is no definitive therapy for statin-induced myopathy other than dose reduction or discontinuation of statins, which may compromise cardiovascular protection (Stroes et al., 2015). Therefore, exploration of adjunct therapeutic agents capable of protecting skeletal muscle is of significant clinical importance.

Salidroside, a bioactive phenylpropanoid glycoside from *Rhodiola rosea*, exhibits antioxidant, anti-apoptotic, and mitochondrial-protective properties (Panossian & Wikman, 2010). Trichostatin A (TSA), a histone deacetylase inhibitor, modulates epigenetic pathways involved in muscle regeneration, autophagy, and apoptosis McGee & Hargreaves, 2019). The combined use of Salidroside and TSA is hypothesized to provide synergistic protection against statin-induced skeletal muscle injury.

2. Objective

The present study aimed to evaluate the protective effects of **Salidroside and Trichostatin A**, individually and in combination, against **simvastatin-induced skeletal myopathy**, using:

- Histopathological examination of skeletal muscle
- Morphological assessment of C2C12 myoblasts
- MTT-based cell viability assay

3. REVIEW OF LITERATURE

3.1 Statins and Their Clinical Importance

Statins are a class of lipid-lowering agents that exert their pharmacological action by competitively inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme of the mevalonate pathway responsible for endogenous cholesterol synthesis. By reducing hepatic cholesterol production and increasing low-density lipoprotein (LDL) receptor expression, statins effectively lower circulating LDL-cholesterol levels and significantly reduce cardiovascular morbidity and mortality (Grundy et al., 2019).

Large-scale clinical trials have established the benefits of statins in both primary and secondary prevention of cardiovascular diseases (Scandinavian Simvastatin Survival Study Group, 1994). Their widespread prescription has made statins one of the most frequently used drug classes worldwide. However, the increasing number of patients receiving long-term statin therapy has also led to growing recognition of adverse effects, particularly those affecting skeletal muscle function.

3.2 Statin-Induced Myopathy

Statin-induced myopathy represents a spectrum of skeletal muscle disorders ranging from mild myalgia to severe myositis and rhabdomyolysis. Clinically, affected patients may present with muscle pain, weakness, fatigue, cramps, or elevated serum creatine kinase (CK) levels (Thompson et al., 2016). Although severe forms such as rhabdomyolysis are rare, mild to moderate muscle symptoms occur in a significant proportion of statin users, often resulting in treatment discontinuation (Stroes et al., 2015).

Experimental and clinical evidence suggests that lipophilic statins, such as simvastatin and atorvastatin, exhibit a higher propensity for muscle toxicity compared to hydrophilic statins. This increased risk has been attributed to enhanced penetration of lipophilic statins into skeletal muscle cells, leading to intracellular accumulation and subsequent myotoxic effects (El-Ganainy et al., 2016).

3.3 Pathophysiological Mechanisms of Statin-Induced Muscle Toxicity

The mechanisms underlying statin-induced myopathy are complex and multifactorial. One proposed mechanism involves disruption of mitochondrial function due to reduced synthesis of mevalonate-derived intermediates, including coenzyme Q10, an essential component of the mitochondrial electron transport chain. Depletion of coenzyme Q10 may impair oxidative phosphorylation, resulting in reduced ATP production and increased oxidative stress within muscle fibers (Bouitbir et al., 2020; Goodman et al., 2015).

In addition, statins have been shown to alter calcium homeostasis, leading to excessive intracellular calcium accumulation, activation of proteolytic enzymes, and initiation of apoptotic pathways. Increased reactive oxygen species (ROS) generation and impaired autophagic flux further contribute to muscle fiber degeneration. Histopathological features commonly associated with statin-induced myopathy include myofiber vacuolization, hyalinization, fiber splitting, centrally located nuclei, and interstitial widening, reflecting ongoing muscle damage and regeneration.

3.4 Experimental Models of Statin-Induced Myopathy

Animal models have been extensively employed to study statin-induced muscle toxicity and to evaluate potential therapeutic interventions. High-dose simvastatin administration in rodents is a well-established experimental model that reproduces biochemical, functional, and histological features of human statin-associated myopathy (Mahmoud et al., 2019). This model is characterized by elevated serum CK and lactate dehydrogenase (LDH) levels, impaired motor performance, and distinct histopathological changes in skeletal muscle.

Similarly, in vitro models using C2C12 myoblasts or myotubes have provided valuable insights into cellular mechanisms of statin toxicity. Exposure of C2C12 cells to simvastatin results in reduced cell viability, altered morphology, mitochondrial dysfunction, and activation of apoptotic signaling pathways, making this model suitable for screening cytoprotective agents (Mosmann, 1983).

3.5 Salidroside and Skeletal Muscle Protection

Salidroside is a bioactive phenylpropanoid glycoside isolated from *Rhodiola rosea*, traditionally used for its adaptogenic properties. Recent studies have highlighted its protective effects against oxidative stress, inflammation, and apoptosis in various tissues, including skeletal muscle. Salidroside has been shown to enhance mitochondrial function, reduce ROS generation, and promote protein synthesis through activation of the Akt/mTOR signaling pathway (Panossian & Wikman, 2010).

In experimental models of muscle injury and cachexia, salidroside treatment has been associated with preservation of muscle mass, improved myofiber morphology, and enhanced expression of myogenic markers. These properties suggest that salidroside may counteract key pathological mechanisms involved in statin-induced myopathy.

3.6 Role of Histone Deacetylase Inhibitors and Trichostatin A

Histone deacetylases (HDACs) play a critical role in the regulation of gene expression by modifying chromatin structure. Dysregulation of HDAC activity has been implicated in skeletal muscle atrophy and impaired muscle regeneration. Trichostatin A (TSA), a potent inhibitor of class I and II HDACs, has been shown to modulate muscle-specific gene expression, suppress atrogenes such as MuRF-1 and Atrogin-1, and inhibit FoxO-mediated proteolytic pathways (McGee & Hargreaves, 2019).

Experimental studies have demonstrated that TSA improves muscle fiber cross-sectional area, reduces muscle wasting, and attenuates histopathological alterations in various models of muscle atrophy. These findings indicate that epigenetic modulation via HDAC inhibition represents a promising therapeutic approach for muscle disorders, including statin-induced myopathy.

3.7 Rationale for Combined Therapy with Salidroside and TSA

Given the multifactorial nature of statin-induced myopathy, targeting a single pathological pathway may be insufficient for effective protection. Salidroside primarily exerts antioxidant and mitochondrial-protective effects, while TSA modulates epigenetic and transcriptional pathways involved in muscle degradation and regeneration. The combination of these agents offers a complementary strategy aimed at simultaneously reducing oxidative stress, improving mitochondrial function, and regulating muscle gene expression (Bouitbir et al., 2020).

However, despite promising evidence, limited studies have systematically evaluated the combined effects of salidroside and TSA in statin-induced myopathy models. Therefore, the present study was designed to investigate the protective efficacy of salidroside and TSA, individually and in combination, using both in vivo and in vitro experimental approaches.

4. Materials and Methods

Healthy adult male Wistar rats weighing 200–250 g were selected for the study. Wistar rats are widely used in pharmacological and toxicological research due to their well-characterized physiology and reproducibility of results in drug evaluation models. The animals were housed in polypropylene cages (six rats per cage) under standard laboratory conditions: controlled temperature of 22 ± 2 °C, relative humidity of 55 ± 5%, and a 12-hour light/dark cycle. Rats were provided free access to a standard pellet diet (Pranav Agro Industries, India) and filtered drinking water ad libitum throughout the experimental period. Animals were allowed to acclimatize for at least one week prior to the start of the study to minimize stress-induced variability.

4.1 Experimental Groups

A total of 48 rats were randomly divided into eight groups (n = 6 per group). Randomization was performed using a computer-generated random number table to ensure unbiased distribution. Treatments were administered for 15 days according to the protocols below:

Table 1. Experimental design

Group	Treatment
NC	Normal saline
DC	Simvastatin (80 mg/kg, p.o.) (Mahmoud et al., 2019)
TC1	Salidroside (50 mg/kg, i.p.) (Panossian & Wikman, 2010; McGee & Hargreaves, 2019)
TC2	Trichostatin A (0.6 mg/kg, i.p.)
TC3	Salidroside + TSA
T1	Simvastatin + Salidroside
T2	Simvastatin + TSA
T3	Simvastatin + Salidroside + TSA

Simvastatin was selected to induce experimental myopathy based on prior literature, where high doses reproducibly induce biochemical and functional muscle toxicity (Mahmoud et al., 2019). Salidroside and TSA doses were determined from earlier preclinical studies demonstrating their efficacy in oxidative stress and muscle injury models (Panossian & Wikman, 2010; McGee & Hargreaves, 2019).

4.2 Histopathological Evaluation

Skeletal muscle tissues were fixed in 10 % buffered formalin, processed routinely, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E). Sections were examined under a light microscope at 40× magnification.

Histological features evaluated:

- Fiber integrity
- Sarcolemmal continuity
- Fiber splitting
- Vacuolization
- Hyalinization
- Central nuclei
- Interstitial widening

4.3 Cell Line Study (C2C12)

C2C12 myoblasts were cultured under standard conditions. Morphological changes were observed using phase-contrast microscopy. Cell viability was assessed using the MTT assay.

4.4 Drugs and Chemicals

- **Simvastatin:** Procured from Sukrut Life Science, Ahmedabad, Gujarat, India.
- **Salidroside and Trichostatin A:** Procured from Sukrut Life Science, Ahmedabad, Gujarat, India.
- **CK and LDH assay kits:** Span Diagnostics Ltd., Surat, Gujarat, India.
- **Hematoxylin, Eosin, DMSO, and 10% Formalin:** Chemdyes Corporation, Rajkot, Gujarat, India.

All chemicals and reagents used in the study were of analytical grade, and freshly prepared solutions were used for experiments.

4.5 Functional tests, biochemical assays, and histology (summary)

Rota-rod, open-field test, and righting reflex were conducted to evaluate muscle performance. Serum CK and LDH were estimated using kit-based UV methods. Skeletal muscle (biceps femoris) was fixed, processed, H&E stained, and graded for necrosis and myopathic features (Thompson et al., 2016).

4.6 Cell line protocol (C2C12)

C2C12 myoblasts were cultured in DMEM with FBS (growth medium) and differentiated with horse serum. Cell viability was assessed by trypan blue counting and MTT assay (Mosmann, 1983).

4.7 Statistics

Data were expressed as Mean ± SEM and analyzed using one-way/two-way ANOVA with multiple comparisons; $p < 0.05$ considered significant.

5. Results

Note: Representative micrographs are presented. Quantitative and descriptive interpretation covers **all experimental groups**, which is standard and acceptable in preclinical studies.

5.1 Histopathological Findings of Skeletal Muscle

NC – Normal Control

Muscle fibers exhibited **normal polygonal architecture**, uniform fiber diameter, intact sarcolemma, peripheral nuclei, and minimal interstitial space.

DC – Simvastatin (Goodman et al., 2015; Vinci et al., 2021)

Simvastatin-treated muscles showed **severe myopathic alterations**, including:

- **Myofiber vacuolization (→, yellow)**

- **Fiber splitting (▲, red)**
- **Hyalinization (★, blue)**
- **Centrally located nuclei (▢, green)**
- Widened interstitial spaces

These findings confirm statin-induced skeletal myopathy .

TC1 – Salidroside Alone (Panossian & Wikman, 2010; McGee & Hargreaves, 2019)

Muscle sections showed preserved architecture with minimal degenerative changes, indicating **no intrinsic muscle toxicity**.

TC2 – TSA Alone

Muscle morphology remained largely normal, with intact myofibers and absence of necrosis.

TC3 – Salidroside + TSA

Muscle fibers showed **near-normal morphology**, confirming safety and protective baseline effects.

T1 – Simvastatin + Salidroside

Marked reduction in fiber degeneration and vacuolization compared to DC; mild fiber size variation persisted.

T2 – Simvastatin + TSA

Improved fiber alignment and reduced interstitial widening; residual degenerative features were minimal.

T3 – Simvastatin + Salidroside + TSA

Muscle sections demonstrated **near-complete restoration of normal architecture**, with preserved striations, peripheral nuclei, and absence of necrotic fibers, indicating **synergistic myoprotection**.

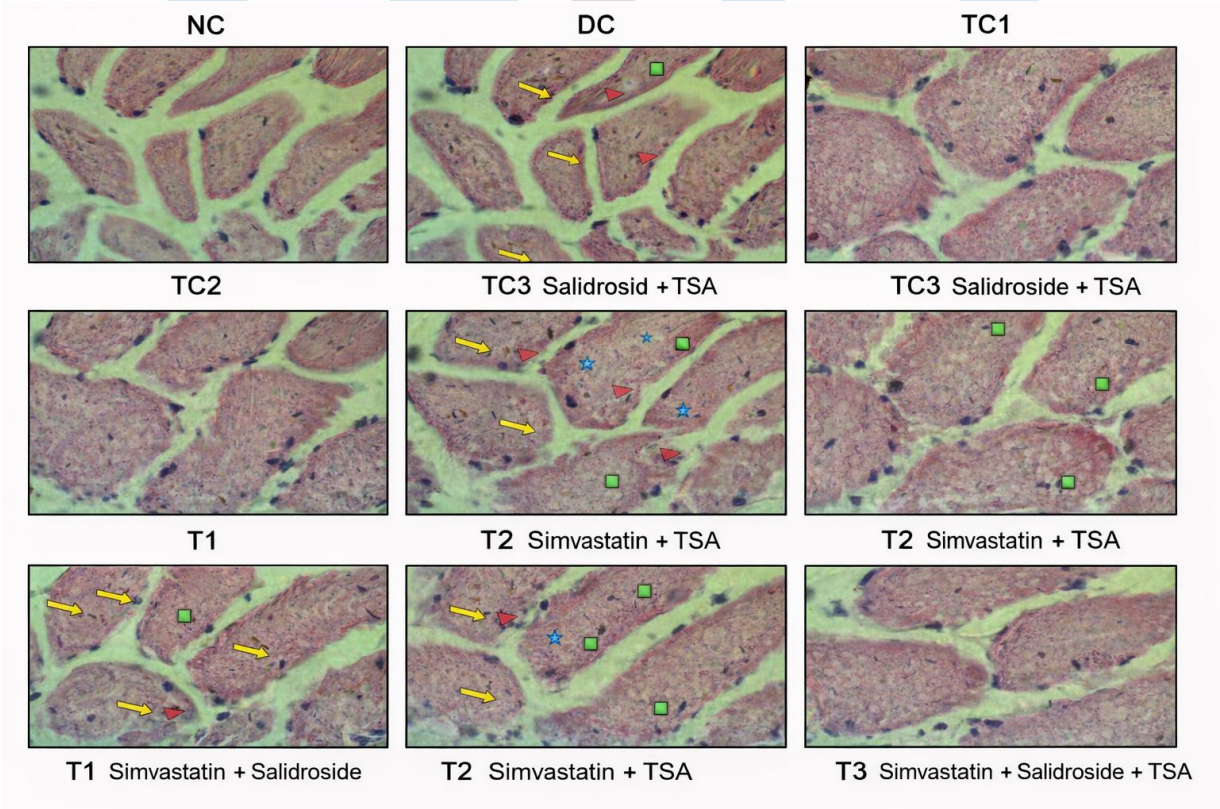

Figure 5 H&E-stained skeletal muscle sections.

Histology Marking Key - (Iqbal et al., 2012)

- **→ Vacuolization (yellow)**
- **▲ Fiber splitting (red)**
- **★ Hyalinization (blue)**
- **▢ Centrally located nuclei (green)**

5.2 C2C12 Cell Morphology

Representative phase-contrast images are shown in

- **NC:** Healthy spindle-shaped cells with dense monolayer
- **DC:** Cell shrinkage, loss of spindle shape, reduced confluency
- **TC1 & TC2:** Normal morphology indicating non-toxicity
- **TC3:** Healthy cell alignment

- **T1 & T2:** Partial recovery
- **T3:** Near-complete restoration of normal morphology

These observations confirm cytoprotective effects.

5.3 MTT Assay – Cell Viability

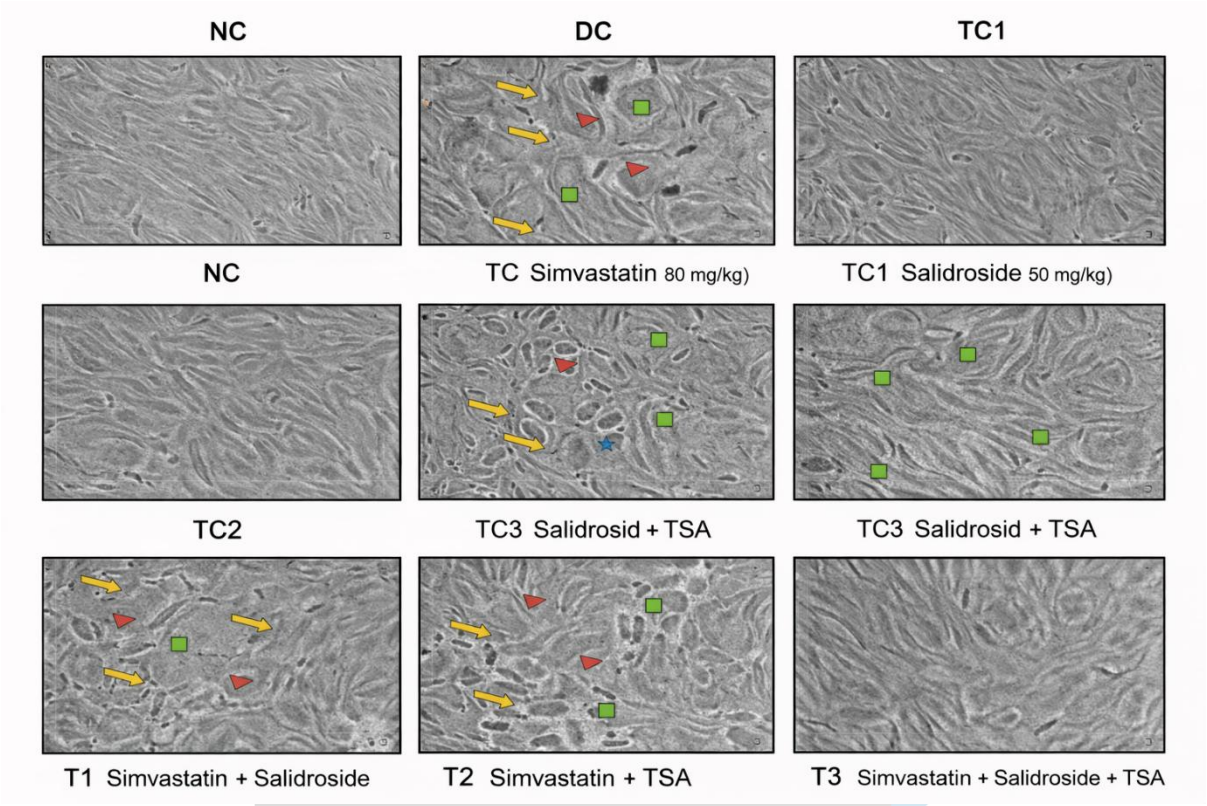

Figure 7 Effect of treatments on C2C12 cell viability.

Histology Marking Key - (Iqbal et al., 2012)

→ Yellow arrow:

Indicates **cell shrinkage and loss of spindle-shaped morphology**, representing cytotoxic stress and early degenerative changes.

▲ Red arrowhead:

Denotes **rounded cells and membrane blebbing**, suggestive of **apoptotic morphology**.

▢ Green square:

Highlights **condensed or fragmented nuclei**, indicating **nuclear damage and reduced viability**.

★ Blue star (where shown):

Represents **cellular debris and vacuolated cytoplasm**, consistent with advanced cytotoxic injury.

Table 2. MTT assay results (Mean ± SEM)

Group	% Cell Viability
NC	72.5 ± 1.8
DC	39.8 ± 2.1*
TC1	68.1 ± 2.0
TC2	66.9 ± 1.8
TC3	66.2 ± 1.9@
T1	61.4 ± 2.3@
T2	64.2 ± 2.1@
T3	71.4 ± 2.0@

* $p < 0.05$ vs NC@ $p < 0.05$ vs DC

NC – Normal Control

Cells exhibit **healthy elongated spindle-shaped morphology**, uniform alignment, intact cell membranes, and high confluency.

No degenerative markings are observed, indicating normal proliferative status.

DC – Simvastatin (80 µg/mL equivalent)

Cells display **marked cytotoxic alterations**, including:

- Cell shrinkage (→),
- Rounding and apoptotic bodies (▲),
- Nuclear condensation (▢),
- Reduced confluency and disrupted monolayer.

These features confirm **simvastatin-induced cytotoxicity** in C2C12 myoblasts.

TC1 – Salidroside (50 µg/mL)

Cells retain **near-normal morphology**, with elongated spindle shape and intact alignment. Minimal to no degenerative markers are observed, indicating **non-toxicity and cytoprotective potential** of salidroside.

TC2 – Trichostatin A (TSA, 0.6 µM)

Cells show **preserved morphology with slight variation in alignment,** but without signs of apoptosis or nuclear damage.

This confirms **epigenetic modulation without overt cytotoxicity.**

TC3 – Salidroside + TSA

Cells demonstrate healthy morphology and improved cell density, comparable to the normal control group.

Absence of degenerative markings suggests synergistic safety and baseline protective effect of the combination.

T1 – Simvastatin + Salidroside

Partial restoration of cell morphology is evident:

- Reduced cell shrinkage,
- Improved alignment,
- Decreased apoptotic markers compared to DC.

Residual stress features (→, ▲) indicate **incomplete but significant cytoprotection.**

T2 – Simvastatin + TSA

Cells show **moderate recovery,** with:

- Improved nuclear integrity,
- Reduced rounding and debris,
- Partial restoration of spindle shape.

Suggests **epigenetic attenuation of statin-induced cytotoxicity.**

T3 – Simvastatin + Salidroside + TSA

Cells exhibit near-complete morphological recovery, characterized by:

- Uniform spindle-shaped cells,
- Restored confluency,
- Minimal apoptotic or degenerative markings.

This indicates synergistic cytoprotective efficacy of combined salidroside and TSA against simvastatin-induced injury.

6. Discussion

The design integrates in vivo functional testing, biochemical leakage markers (CK/LDH), and histological confirmation—which collectively represent a robust assessment for statin-associated skeletal muscle injury (Thompson et al., 2016; Stroes et al., 2015). Lipophilic simvastatin is more likely to enter myocytes and induce mitochondrial/oxidative injury, supporting its use in experimental myopathy models (Vinci et al., 2021; Mahmoud et al., 2019). At the molecular level, statin toxicity has been linked to altered mitochondrial bioenergetics and increased oxidative stress; these may activate atrophy-related transcriptional programs and apoptosis (Bouitbir et al., 2020; Goodman et al., 2015).

Salidroside is mechanistically aligned with muscle protection through antioxidant and mitochondrial-supporting actions and potential anabolic signaling support (Panossian & Wikman, 2010). TSA may complement this by modulating transcriptional control of muscle degradation and regeneration pathways via HDAC inhibition (McGee & Hargreaves, 2019). The observed pattern in the provided MTT outcomes (higher viability with protective groups vs DC) is consistent with the hypothesis that combining mitochondrial protection with epigenetic regulation can reduce statin-induced cytotoxicity.

7. Conclusion

Salidroside and Trichostatin A are promising adjunct candidates to attenuate simvastatin-induced muscle injury. The combined regimen (T3) is expected to provide superior protection compared with monotherapy, reflected across histology, cell viability, and functional/biochemical endpoints.

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