Effects of Chinese herbal medicine B307 supplementation on muscle endurance and recovery after exhaustive swimming in ICR mice

Tai-Yuan Chuang
National Taiwan Normal University
Chia-Ying Lien
National Taiwan University of Physical Education and Sport
Ya-Chun Tsai
National Tsing Hua University - Nanda Campus
Kuei-Fu Lin
National Tsing Hua University - Nanda Campus
Chih-Hsiang Hsu
National Taiwan Normal University
Wen-Jhen Wu
National Taiwan Normal University
Li-Yu Su
National Taiwan Normal University
Chen-Wen Lu
National Taiwan Normal University
Chung-Hsin Wu (megawu@ntnu.edu.tw)
National Taiwan University Department of Life Science https://orcid.org/0000-0002-4470-2448

Research

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Authors: Tai-Yuan Chuang[1,2], Chia-Ying Lien[2], Ya-Chun Tsai[3], Kuei-Fu Lin[3], Chih-Hsiang Hsu[1], Wan-Jhen Wu[1], Li-Yu Su [1], Chen-Wen Lu[1], and Chung-Hsin Wu[1,*]

Affiliation: [1] School of Life Science, National Taiwan Normal University, Taipei City, Taiwan

[2] Department of Athletics, National Taiwan University, Taipei City, Taiwan

[3] Department of Physical Education, National Tsing Hua University, Hsinchu City, Taiwan

***Corresponding author:**

Chung-Hsin Wu

Professor of Department of Life Sciences, National Taiwan Normal University

Tel: +886-2-77346363, Fax: +886-2-29312904, E-mail: megawu@ntnu.edu.tw

ADD: School of Life Science, No. 88, Sec 4, Ting-Chow Rd, Taipei, Taiwan, 116, R.O.C.

ABSTRACT:

Background: Exhaustive exercise may damage muscles due to oxidative stress and inflammation and cause muscle fatigue and soreness. The study investigated the effects of Chinese herbal medicine (CHM) B307 supplementation on muscle endurance and recovery after exhaustive swimming (ES) in ICR mice.

Methods: All mice were subjected to ES in the form of a forced swimming test. Thirty-two male ICR mice were randomly divided into 4 groups: Sham + ES, CHM B307 pretreatment + ES (Pre+ES), CHM B307 posttreatment + ES (Post+ES), and CHM B307 dual treatment + ES (Dual+ES). Using immunohistochemistry, chemiluminescence, and biochemical analysis, muscular endurance, fatigue, oxidative stress, inflammation, and damage were examined and compared among the groups.

Results: Our results revealed that the Pre+ES and Dual+ES groups had remarkably better muscular endurance during ES than the Sham+ES and Post+ES groups. In addition, the Pre+ES, Post+ES, and Dual+ES groups had significantly alleviated fatigue, oxidative stress, inflammation, and damage after ES compared with the Sham+ES group.

Conclusion: CHM B307 can be a protective supplement against damage from exhaustive exercise because of its antifatigue, antioxidation, anti-inflammation, and antidamage functions.

Keywords: Chinese herbal medicine, Exhaustive swimming, Muscular endurance Oxidative stress, Inflammation, Gastrocnemius muscles, ICR mice

Introduction

Humans who participate in acute endurance exercise often increase their oxygen consumption, which produces reactive oxygen species (ROS) and causes muscle damage [1–3]. ROS may play a crucial role in body defense systems [4], but excessive ROS contributes to diseases and conditions such as diabetes, cancer, cardiovascular disease, and neurological disorders, even affecting aging processes [5, 6]. Endogenous antioxidant capacity also reportedly increases following exercise, which can partially attenuate oxidative stress by scavenging ROS [7–10]. Even though individuals can remove ROS from their bodies, intense or prolonged exercise generates a considerable number of ROS, far more than our antioxidant capacity [11]. As a result, excessive ROS causes oxidative stress because of an imbalance between oxidants and antioxidants in favor of oxidants. Many sports science studies have indicated that skeletal muscle fibers exposed to a high concentration of ROS may lead to impaired muscle force production and fatigue [12–14].

Athletes who engage in exhaustive exercise with limited recovery periods may see their athletic performance decline and muscle fatigability increase subsequently [15]. Excessive exercise can affect the balance of hormone adjustment and cause oxidative stress and subsequent cell membrane damage in both male and female rats [16]. To examine physiological damage induced by exhaustive exercise, forced swimming is widely used in studies of exhaustive exercise testing in rats [17]. In forced swim tests, exercise intensity can be indirectly graded by the addition of a weight load attached to the body or tail to decrease the time until exhaustion [18]. Thus, forced swim tests provide an efficient animal model for studying exercise-induced physiological damage.

Unlike health-promoting exercise, athletes must undergo severe training daily to improve their athletic performance; therefore, identifying an appropriate sports supplement to protect muscles against oxidative stress and inflammation is essential. It was reported that *Danggui Buxue Tang* has a favorable antifatigue effect in male mice after forced swimming [19]. Thus, Chinese herbal medicines (CHMs) may provide

natural food sources of sports supplements for achieving antifatigue effects. To our knowledge, however, the antiantioxidant, anti-inflammatory, and muscle-relieving effects of CHMs after forced swim tests have rarely been elucidated. As we have previously suggested, CHM B307 can effectively enhance muscle strength in mice [20, 21]. B307 may be an appropriate sports supplement to protect muscle against oxidative stress and inflammation for athletes training under a high exercise intensity. Therefore, the purpose of this study was to examine the antiantioxidant, anti-inflammatory, and muscle-relieving effects of CHM B307 in Institute of Cancer Research (ICR) mice after an exhaustive swimming (ES) test.

Materials and Methods

Animal preparation

Thirty-two male ICR mice were purchased from BioLASCO Taiwan Yi-Lan Breeding Center (fully accredited by AAALAC International). In accordance with the Institutional Guidelines of the Animal Care and Use Committee of National Taiwan Normal University (NTNU), the mice were maintained in the animal facility of NTNU under specific pathogen-free conditions. All animal experiments were approved by the Institutional Animal Care and Use Committee of our university (Protocol number: NTNU Animal Experiments No. 108011). All the mice were housed in an environment with a constant temperature of 22 °C ± 2 °C and subjected to a 12-h light/dark cycle, and the mice had *ad libitum* access to water and food.

Study design

The study assessed the effects of Chinese herbal medicine (CHM) B307 supplementation on muscle endurance and recovery after ES in ICR mice. As suggested in Figure 1, all mice were subjected to forced ES executed under a forced swimming test. A total of 32 male ICR mice were randomly divided into 4 groups: Sham + ES, CHM B307 pretreatment + ES (Pre+ES), CHM B307 posttreatment + ES (Post+ES), and CHM B307 dual treatment + ES (Dual+ES). The Pre+ES mice were orally given B307

97 treatment before ES, Post+ES mice were orally given B307 treatment after ES, Dual+ES
98 mice were orally given B307 treatment both before and after ES, and the Sham+ES
99 group was given a normal diet and drinking water before and after ES. The experiment
100 was approved by the Institutional Animal Care and Use Committee of NTNU (protocol
101 number NTNU Animal Experiments No. 108011).
102
103 **ES experiment design**
104 As suggested in [22], a weight-loaded ES procedure was carried out in a swimming
105 tank (50 × 50 × 50 cm^3) with 30-cm-deep water maintained at 25 ± 3 °C for 1 h after the
106 oral administration of CHM B307. Then mice were removed from the pool, dried with a
107 paper towel, and returned to their original cages. The pool water was replaced after
108 each session. ES was determined by observing the loss of coordinated movements and
109 failure to swim. ES time was recorded immediately when mice were completely
110 exhausted and failed to return to the surface to breathe within 5 s.
111
112 **Oral treatment of CHM B307**
113 CHM B307 is a commercially available nutritional supplement (Sun-Ten Pharmaceutical
114 Company, New Taipei City, Taiwan). The Pre+ES, Post+ES, and Dual+ES groups were
115 orally treated with CHM B307 extract. We used a gavage tube to orally administer CHM
116 B307 extract (50 mg/mL; the pH value was approximately 7.0) and its vehicle (dimethyl
117 sulfoxide). Doses were adjusted according to the individual weight of the mouse. The
118 feeding frequency was 4 times for the Pre+ES and Post+ES mice and 8 times for the
119 Dual+ES mice at intervals of 12 hours. The dosage and administration of CHM B307
120 extract were much lower than the median lethal dose (LD_{50}). The sham group was
121 orally treated with the vehicle.
122
123 ***Chromatographic fingerprint analysis of CHM B307***
124 All CHM B307 chemicals were solubilized in distilled H_2O/MeOH and then analyzed
125 using cetonitrile of high-performance liquid chromatography grade (Burdick & Jackson,

Gyeonggi-do, Korea) and methanol (Avantor, Center Valley, PA, USA). Purified water from a Milli-Q water purification system (EMD Millipore, Billerica, MA, USA) was used to dissolve the ingredients.

Urine and blood tests

After the final ES procedure, mice were immediately sacrificed under mild anesthesia. Urine was collected from the bladder, and blood was collected from the abdominal aorta into centrifuge tubes using a heparinized syringe. The urine samples were analyzed using 10 parameter urinalysis reagent strips for urine tests (Medisave Ltd., Weymouth, UK). The blood samples were analyzed using a blood smear, as is common practice to evaluate the health status of an animal. The blood samples were taken in a blood sampling tube with heparin (Terumo Inc., Tokyo, Japan) and centrifuged for 10 min at 1,000 × g at 4 °C to separate the plasma, which was stored at −80 °C until analysis. The plasma samples were analyzed for lactate acid using an assay kit (Cayman Chemical, MI, USA). To determine blood ROS levels, lucigenin- and luminol-amplified chemiluminescence (CL) methods were used to measure $O2^{\bullet-}$ and H_2O_2 activity. The lucigenin-enhanced CL method is reliable for ROS assays. A heparinized sample (0.2 mL) of whole blood was taken from the left femoral artery of each mouse. ROS blood levels were measured using a CL analyzer (CLA-ID3 chemiluminescence analyzer; Tohoku Electronic Industrial Co., Ltd., Sendai, Japan) after the addition of 1.0 mL of 0.1 mM lucigenin in phosphate-buffered saline (pH 7.4) to the samples. The assay was duplicated for each sample, and total CL counts in 600 s were calculated by integrating the area under the curve.

Muscle immunohistochemistry analysis

ICR mice were anesthetized and then cardiac-perfused with phosphate-buffered saline containing 4% formaldehyde (EM grade glutaraldehyde solution, Sigma-Aldrich St. Louis, MU, United States). Gastrocnemius muscle tissue was then removed and fixed with 4% formaldehyde (EM grade). The muscle specimens were embedded in paraffin

and cut into tissue sections with a thickness of 5 μm. The tissue sections were mounted on slides for histological and muscle immunohistochemistry (IHC) analysis. Using the heat-induced epitope retrieval method, the sections were separately stained at room temperature for 1 h with antibodies of superoxide dismutase 2 (SOD2; Cat. #13141, Cell Signaling Technology Inc., Danvers, MA, USA), tumor necrosis factor alpha (TNF-a) (Cat. #11948, Cell Signaling Technology Inc.), nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) (Cat. #3034, Cell Signaling Technology Inc.), and vinculin (Cat. #13901, Cell Signaling Technology Inc.) immunostaining controls for each antibody. Serial 5-μm cross-sections were treated with the unanimous staining protocol. Immunostaining detection was executed using incubation with biotinylated secondary antibodies (NovolinkTM polymer detection system I) at room temperature for 30 min and then by incubation with avidin–biotin–HRP complex (NovolinkTM polymer detection system I) for an additional 30 min. Immunostaining visualization was performed with DAB Chromogen (NovolinkTM polymer detection system I) and counterstained with hematoxylin (NovolinkTM polymer detection system I) following the supplier's protocol.

Muscle Western blot analysis

The removed gastrocnemius muscle tissue was kept in a buffer solution to maintain its pH for a Western blot analysis. Skeletal muscle protein was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane. We used SOD2 antibodies (Cat. #13141, Cell Signaling Technology Inc.) to identify expression levels of anti-oxidative proteins in the skeletal muscle tissue by means of a horseradish peroxidase–linked secondary antibody. In addition, Enhanced chemiluminescence Western blotting detection reagents (GE Healthcare Life Sciences, Piscataway, NJ, USA) were used to make immunoreactive bands perceptible. An ImageQuant LAS-4000 biomolecular imager (GE Healthcare Life Sciences) was used to detect chemiluminescence. Image J software (version 1.48t, Wayne Rasnabd, USA) was used to count densitometric assessments of the bands.

Statistical analysis

The data in Figures 3, 4, and 6 were obtained from at least 3 independent experiments. Values for the data were expressed as mean ± standard error of the mean (SEM). Differences among the ICR mice groups were evaluated using a two-way analysis of variance (ANOVA). If a significant *F*-value was observed, the Student–Newman–Keuls multiple comparisons posttest was conducted to determine where differences existed. *P* values of <0.05 were considered significant.

Results

Chromatographic fingerprint of CHM B307

Chromatographic fingerprint analysis of CHM B307 are shown in Figure 2. Ginseng (*panax ginseng Radix*), schizandra (*schizandrae Fructus*), ophiopogon (*ophiopogonis Tuber*), and danshen (*salviae miltiorrhizae Radix*) are the main herbal ingredients in CHM B307. A bioactive marker substance for *ginseng Radix* is ginsenoside Rb1; marker substances for *schizandrae Fructus* are schizandrin and gomisin A; a marker substance for *ophiopogonis Tuber* is methylophiopogonanone B; and marker substances for *salviae militiorrhizae Radix* are rosmarinic acid, salvianolic acid B, and tanshinone IIA.

Effects of oral CHM B307 treatment on the body weight and ES time of mice

CHM B307 is related to cardiac function and skeletal muscle strength in mice [20, 21]. We sought to determine whether any change in muscle endurance to ES occurred with oral CHM B307 treatment. We measured the body weight and ES time of ICR mice after oral CHM B307 treatment. After ES, no significant difference was observed in body weight in any group ($p > 0.05$, Fig. 3A). However, as presented in Figure 3B, the Pre+ES and Dual+ES groups had ES times 250% longer than those of the Sham+ES and Post+ES groups ($p < 0.01$, Fig. 3B). The results suggest that CHM B307 pretreatment promoted muscle endurance in the mice.

Effects of oral CHM B307 treatment on pathological factors in urine and blood after ES

We examined the effects of oral CHM B307 treatment in inflammatory factors in urine and blood. Figure 4A presents a comparison of leukocyte counts in urine among the groups. Leukocyte counts in the urine of the Sham+ES mice were significantly increased after ES, whereas the leukocyte counts of the Pre+ES, Post+ES, and Dual+ES mice were significantly decreased ($P < 0.01$, Fig. 4A). We also compared neutrophil counts in blood samples (Fig. 4Ba). Compared with normal mice, neutrophil counts for the Sham+ES mice were significantly increased after ES, and neutrophil counts for the Pre+ES, Post+ES, and Dual+ES mice were significantly decreased ($P < 0.01$, Fig. 4Ba).

To examine the effects of oral CHM B307 treatment on muscle fatigue, we compared lactic acid levels in the blood of the mice (Fig. 4Bb). Lactic acid levels of the Sham+ES mice were significantly increased after ES, whereas those of the other groups were significantly decreased ($p < 0.01$).

Effect of oral CHM B307 treatment on oxidative stress in muscle tissue after ES

To examine the effects of oral CHM B307 treatment on oxidative stress in muscle tissue after ES, we evaluated expression levels of SOD2 in gastrocnemius muscles using IHC and biochemical analysis. As indicated in Figure 5, SOD2 expression in the Pre+ES, Post+ES, and Dual+ES mice was markedly higher than in the Sham+ES mice. Western blotting (Fig. 6Aa) revealed SOD2 expression in the treated mice was significantly greater than that in the Sham+ES mice ($p < 0.01$–0.05, Fig. 6Ab), Notably, SOD2 expression in the Dual+ES mice was significantly higher than in the Pre+ES and Post+ES mice ($p < 0.05$, Fig. 6Ab).

We further examined blood ROS expression among the 4 groups through chemiluminescence analysis, because SOD2 can clear mitochondrial ROS. Figure 6Ba shows that the expression of blood ROS in the treated mice was lower than in the Sham+ES mice, and the expression of blood ROS in the Dual+ES mice was lower than in the Pre+ES and Post+ES groups. Figure 6Bb shows that quantified blood ROS expression in gastrocnemius muscle tissue of the Pre+ES, Post+ES, and Dual+ES mice was

significantly lower than in the Sham+ES mice ($P < 0.01–0.05$, Fig. 6Bb). Notably, quantified blood ROS expression in the Dual+ES mice was significantly lower than in the Pre+ES and Post+ES groups ($p < 0.01$).

Effect of oral B307 treatment on muscle tissue inflammation after ES

To examine the effects of oral CHM B307 treatment on muscle tissue inflammation after ES, we compared expressions of TNF-a and NFκB in the gastrocnemius muscles of mice after ES. As revealed by IHC staining (Fig. 7), TNF-a expression in the treated mice was markedly weaker than in the Sham+ES mice. Furthermore, as Figure 8 shows, NFκB expression in the treatment groups was obviously weaker than in the Sham+ES group.

Effect of oral B307 treatment on muscle tissue damage after ES

In mammalian cells, vinculin plays a role in muscle repair systems. Thus, we compared vinculin expression in gastrocnemius muscle tissue among the groups through IHC staining. As shown in Figure 9, vinculin expression in the treated mice was obviously stronger than in the sham-treated mice after ES. The results demonstrate that CHM B307 modulated focal adhesion structure in skeletal muscle after ES.

Discussion

The use of CHM as a sports supplement is becoming more common. Many agree that the health benefits of plants are attributable to bioactive compounds that have several physiological effects on the human body. Many CHMs are used to enhance muscle strength and body mass. The purpose of this study was to provide evidence that CHM B307 has considerable potential as a sports supplement.

As suggested by chromatographic fingerprint analysis of CHM B307 (Fig. 2), ginseng and danshen are its main herbal ingredients. Many studies have reported that ginseng has antioxidant [23] and anti-inflammatory [24,25] properties. Others have reported that ginseng can improve alertness and fatigue resistance through cortisol stimulation [26]. Danshen can alleviate heart disease and ameliorate the effects of

atherosclerosis in humans [27, 28] and rodents [29]. As suggested in a review paper [30], ginseng has been used as an endurance performance enhancer. Alkaloid supplementation can improve athletic performance for intense sprinting and cycling exercises. Some other alkaloids such as green tea extracts can increase body mass and improve muscle composition in athletes.

According to the results of this study, oral CHM B307 treatment can significantly decrease leukocyte counts in the urine and neutrophils counts in the blood of mice after ES (Fig. 4A, Ba). In blood cells, 5 types of leukocyte exist, of which neutrophils are the most abundant. Neutrophils can mediate acute inflammatory tissue damage because they can act against infectious microbes and produce ROS to kill invading microorganisms [31]. After exhaustive exercise, neutrophil activation can result in the overproduction of ROS, which contributes to muscle injury and leads to oxidative stress [32, 33]. Our results indicate that mice largely increase their blood ROS production after ES, but oral CHM B307 treatment can significantly decrease ROS production (Fig. 6B). Nitochondrial SOD2 plays an essential role in endogenous antioxidant capacity, which can inhibit oxidative stress. Our study demonstrated that oral CHM B307 treatment may increase SOD2 expression in gastrocnemius muscle tissue in mice after ES (Figs. 5, 6A). Oral CHM B307 treatment may increase endogenous antioxidant capacity after ES, which can partially attenuate neutrophil activation and oxidative stress. The role of CHM B307 supplements in reducing exercise-induced oxidative stress can enhance muscle recovery and energy maintenance during intensive exercise in athletes. Therefore, we believe that CHM B307 supplements could be an ideal candidate as a sports supplement for enhancing muscle performance.

During intense exercise, lactic acid is produced in muscles and then accumulated, which can lead to muscle fatigue and pain. Our findings indicate that lactic acid levels in the blood of mice after ES considerably increase compared with normal mice, but these levels were significantly decreased in these mice with oral CHM B307 treatment (Fig. 4Bb). Though the possible role of CHM B307 supplements in reducing lactic acid is unclear, we suggest that CHM B307 is an ideal candidate for alleviating muscle fatigue

and pain after intensive exercise. In addition, neutrophil activation mediates pathological processes and acute inflammatory tissue damage. Acute exercise may activate the NFκB-TNFa inflammatory signaling pathway in rat skeletal muscles [34]. TNF-a has been associated with muscle inflammation that may be mediated by ROS and NFκB, both of which upregulate ubiquitin/proteasome activity [35]. TNF-a and NFκB play key roles in muscle inflammation after exhaustive exercise. Our results demonstrate that oral CHM B307 treatment can decrease the expression of TNF-a and NFκB in murine gastrocnemius muscles after ES (Figs. 7 and 8). In mammalian cells, vinculin is a membrane-cytoskeletal protein involved in the linkage of adhesion molecules to actin cytoskeleton [36]. Our study demonstrates that oral CHM B307 treatment can effectively alleviate muscle injury in mice after ES by promoting the expression of membrane-cytoskeletal vinculin protein (Fig. 9). We therefore conclude that CHM B307 could be an ideal sports supplement after exhaustive exercise because of oxygen debt remission, NFκB-TNFa inflammatory signaling reduction, and injury recovery in muscle tissue.

Conclusions

Our studies demonstrate that oral treatment of CHM B307 can effectively strengthen muscle endurance by prolonging ES time (Fig. 3B), mitigating systemic inflammatory response by reducing the number of leukocytes in urine and blood (Fig. 4A, Ba), reducing muscle oxygen debt though reducing lactic acid production (Fig. 4Bb), attenuating muscle oxidative stress by enhancing expression of anti-oxidative SOD2 protein (Figs. 5, 6A), reducing ROS production (Fig. 6B), alleviating muscle inflammation by suppressing expression of inflammatory TNF-a and NFκB proteins (Figs. 7, 8), and recovering muscle damage by promoting expression of membrane-cytoskeletal vinculin protein (Fig. 9). Our study indicates that CHM B307 could be an ideal candidate for a sports supplement that it should be taken before and after exhaustive exercise because of its antifatigue, antioxidation, anti-inflammation, and antidamage effects in skeletal muscles.

Abbreviations

CHM: Chinese herbal medicine; ES: Exhaustive swimming; Pre+ES: Pretreatment + ES; Post+ES: Posttreatment + ES; Dual+ES: Dual treatment + ES; ROS: Reactive oxygen species; ICR: Institute of Cancer Research; LD_{50}: Median lethal dose; CL: Chemiluminescence; IHC: Immunohistochemistry; SOD2: Superoxide dismutase 2; TNF-a: Tumor necrosis factor alpha; NFκB: Nuclear factor kappa-light-chain-enhancer of activated B cells; SEM: Standard error of the mean; ANOVA: Analysis of variance

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Availability of data and supporting materials section.

Please contact corresponding author for additional data.

Authors' contributions

TY, CY, YC: randomization of the protocol training of animals, and animal training assistance; KF, CH: literature review; WJ, LY, CW: molecular biology assays; CH: dissertation guidance, interpretation of the data and drafted the manuscript; All authors read and approved the final manuscript.

Authors' information

TYC: Associate Professor of Department of Athletics, National Taiwan University (hbeaverk@ntu.edu.tw); CYL: Associate Professor of Department of Athletics, National Taiwan University (chiayinglien@ntu.edu.tw); YCT: Master of Department of Physical Education, National Tsing Hua University (alex84020934@gmail.com); KFL: Professor of Department of Physical Education, National Tsing Hua University (steve@mail.nd.nthu.edu.tw); CHH: Postdoctoral of School of Life Science, National Taiwan Normal University (jerrycarry.tw@gmail.com); WJW: Research Assistant of School of Life Science, National Taiwan Normal University (efgy78@gmail.com); LYS: Research Assistant of School of Life Science, National Taiwan Normal University (julia10025@gmail.com); CWL: Postdoctoral of School of Life Science, National Taiwan Normal University (kumo.lu@gmail.com); CHW: Professor of School of Life Science, National Taiwan Normal University (megawu@ntnu.edu.tw)

Ethics Approval and Consent to Participate

Ethical approval was obtained from the Institutional Animal Care and Use Committee of National Taiwan Normal University (Protocol number: NTNU Animal Experiments No. 108011).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Author details

[1] School of Life Science, National Taiwan Normal University, Taipei City, Taiwan. [2] Department of Athletics, National Taiwan University, Taipei City, Taiwan. [3] Department of Physical Education, National Tsing Hua University, Hsinchu City, Taiwan

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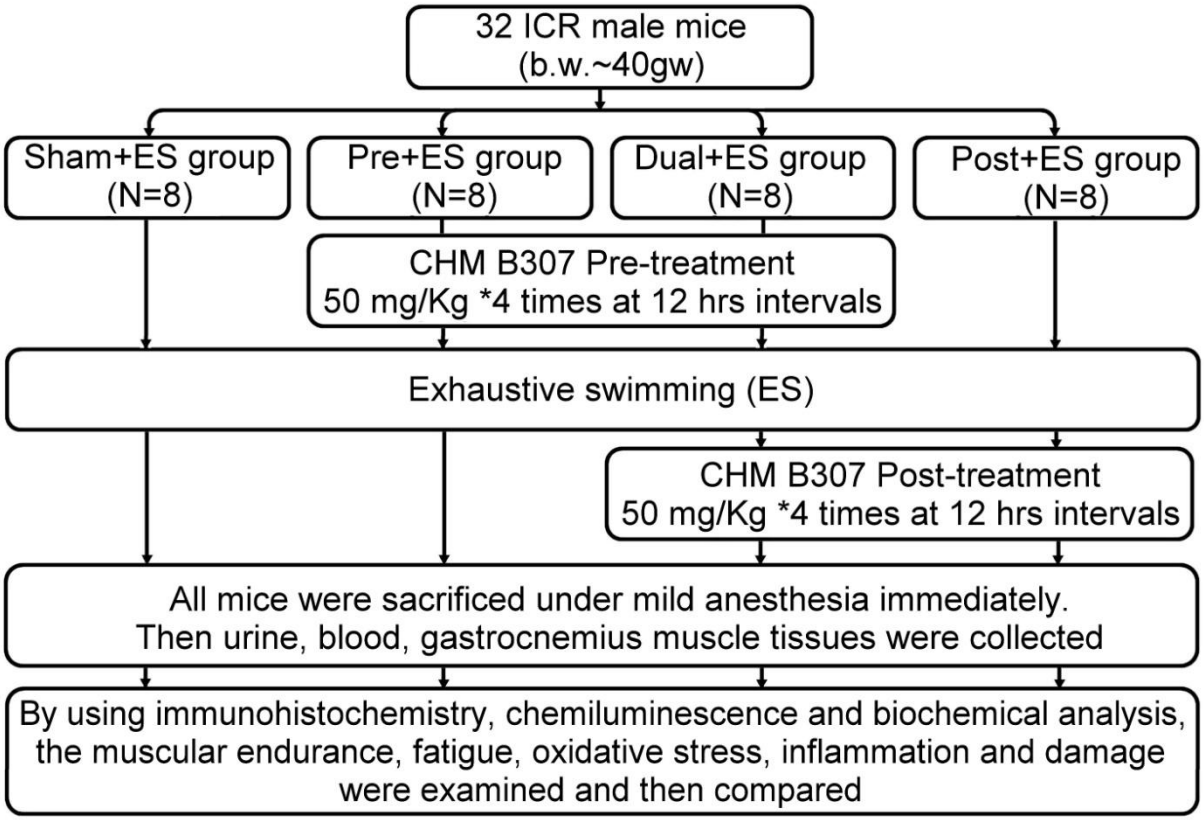
485 **Figure legends**



486

487 **Fig. 1: Study design for assessing the effects of supplemental Chinese herbal medicine**
488 **B307 in ICR mice after exhaustive swimming (ES).** Mice were subjected to ES under a
489 forced swimming test. Thirty-two male ICR mice were randomly divided into 4 groups:
490 Sham + ES, CHM B307 pretreatment + ES (Pre+ES), CHM B307 posttreatment + ES
491 (Post+ES), and CHM B307 dual treatment + ES (Dual+ES).

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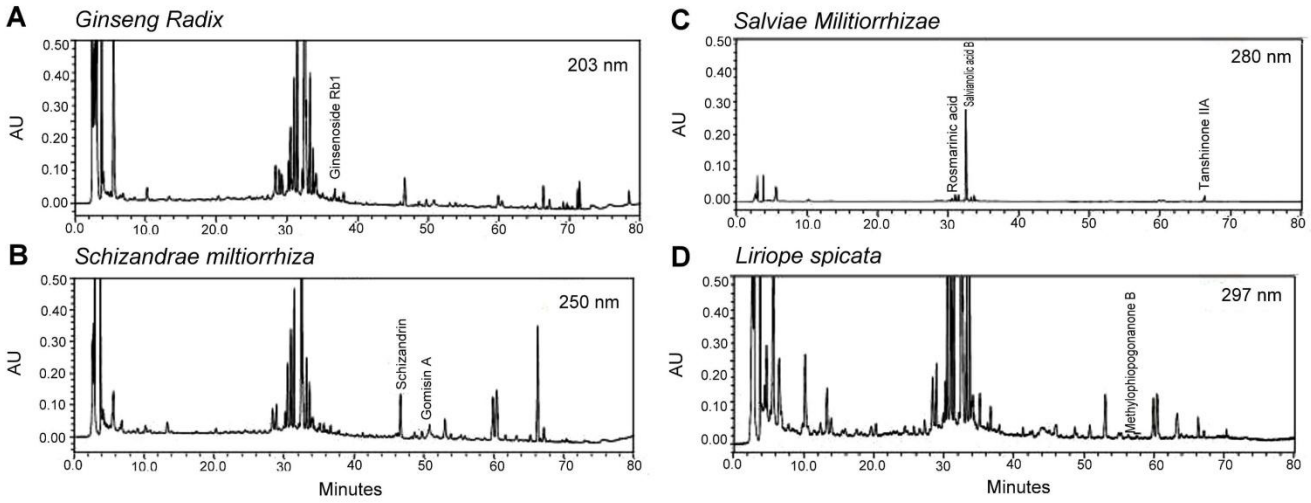

493

494 **Fig. 2: Chromatographic fingerprints of the Chinese herbal formula B307 from**
495 **high-performance liquid chromatography.** The peak for bioactive marker substances
496 for (A) *Ginseng Radix* was ginsenoside Rb1(λ = 203 nm); (B) peaks for *Schizandrae*
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499 nm); and (D) the peak for *Liriope spicata* was methylophiopogonanone B (λ = 297 nm).
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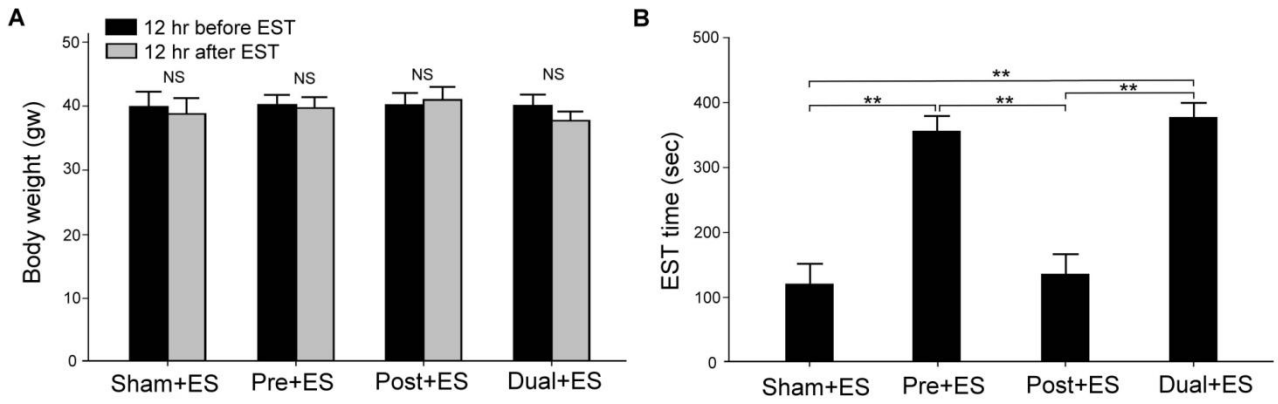


Fig. 3: Comparisons of the body weight and ES time of mice with and without B307 treatment. (A) Statistical comparison of quantified body weight 12 h before and after the ES test. No differences (paired t test, $p > 0.05$) were noted among the Sham+ES, Pre+ES, Post+ES, and Dual+ES groups. (B) Statistical comparison of quantified ES time among groups. The data indicated that the ES times of Pre+ES and Dual+ES mice was significantly longer than those of Sham+ES and Post+ES mice (**$P < 0.01$, one-way ANOVA followed by Student–Newman–Keuls multiple comparison posttest). All data are shown as mean ± SEM.

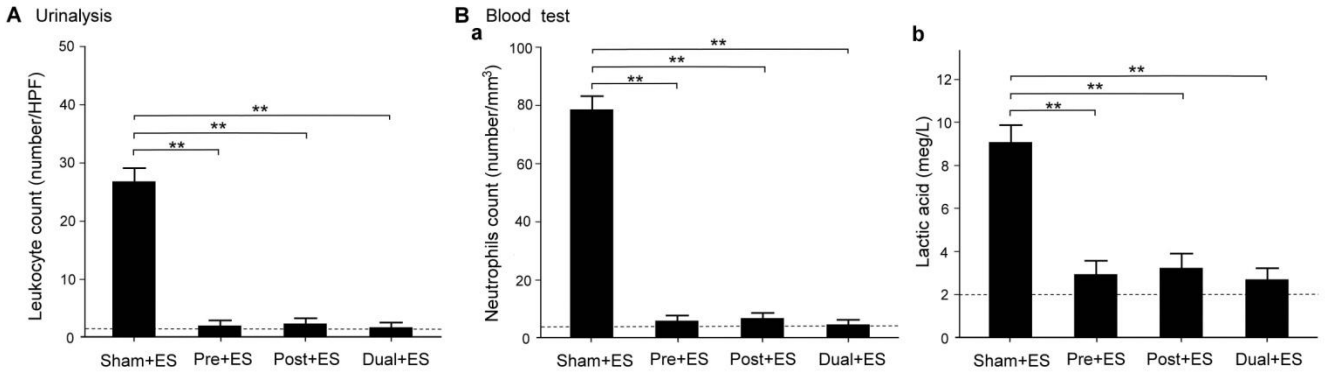


Fig. 4: Comparisons of urine and blood tests for mice with and without CHM B307 treatment. (A) Statistical comparison of quantified leukocyte counts in urine among the groups. Leukocyte counts of Pre+ES, Post+ES, and Dual+ES mice were significantly lower than those of Sham+ES mice ($p < 0.01$). (B) Statistical comparison of quantified neutrophil counts and lactic acid in the blood of the 4 groups. Neutrophils counts and lactic acid levels in the Pre+ES, Post+ES, and Dual+ES mice were significantly decreased ($p < 0.01$). The dashed lines represent averaged values in normal mice. All data are shown as mean ± SEM (**$P < 0.01$, two-way ANOVA followed by Student–Newman–Keuls multiple comparison posttest). Eight experiments were conducted for each treatment.

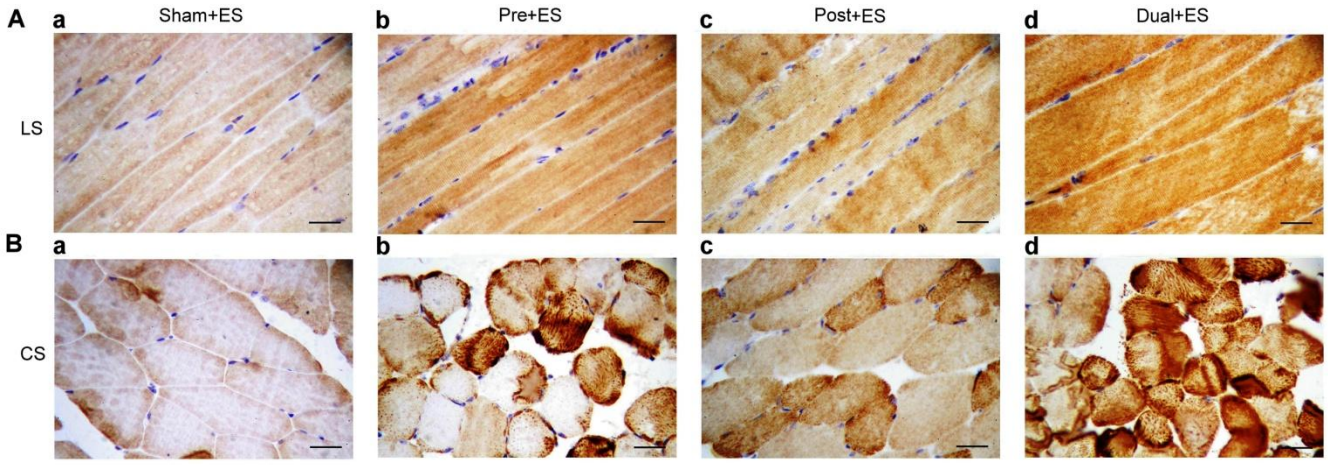


Fig. 5: Representative immunohistochemistry-based SOD2 expressions of gastrocnemius muscle tissue between mice with and without Chinese herbal medicine B307 treatment. (A) Longitudinal sections and (B) cross-sections of gastrocnemius muscle tissue obtained through immunohistochemistry staining indicate that SOD2 expression (expressed by a deep brown color) in Pre+ES, Post+ES, and Dual+ES mice (b–d) was substantially higher than in Sham+ES mice (a). Scale bar = 30 μm.

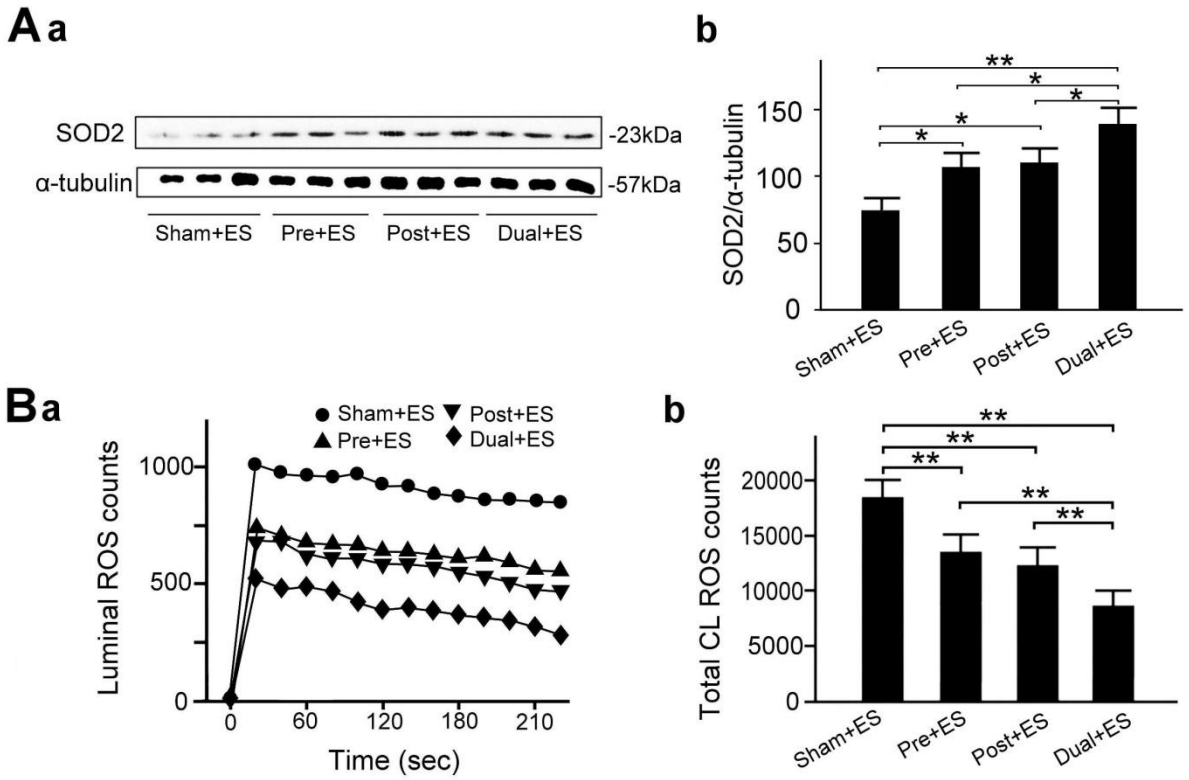


Fig. 6. Comparisons of muscle SOD2 and blood reactive oxygen species (ROS) expression between mice with and without B307 treatment. (A) SOD2 expression of gastrocnemius muscle tissue among the Sham+ES, Pre+ES, Post+ES, and Dual+ES groups determined through Western blotting (a). Statistical comparison of quantified SOD2 expression among the groups (b). (B) Blood ROS expression among the groups determined through chemiluminescence analysis (a). Statistical comparison of quantified blood ROS expression among the groups (b). All data are shown as mean ± SEM (**$P < 0.01$, *$P < 0.05$, two-way ANOVA followed by Student–Newman–Keuls multiple comparison posttest). Experiments were repeated 3 times for each treatment.

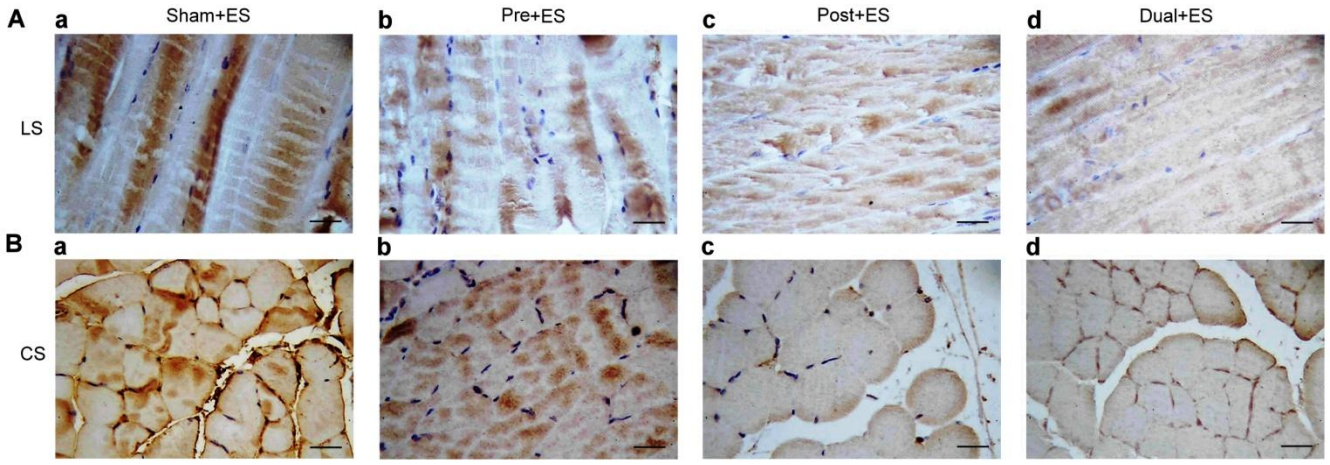


Fig. 7: Representative immunohistochemistry-based TNF-a expression of gastrocnemius muscle tissue in response to ES between mice with and without B307 treatment. (A) Longitudinal sections and (B) cross-sections of gastrocnemius muscle tissue; TNF-a expression (expressed by a deep brown color) in Pre+ES, Post+ES, and Dual+ES mice (b–d) was markedly weaker than in Sham+ES mice. Scale bar = 30 µm.

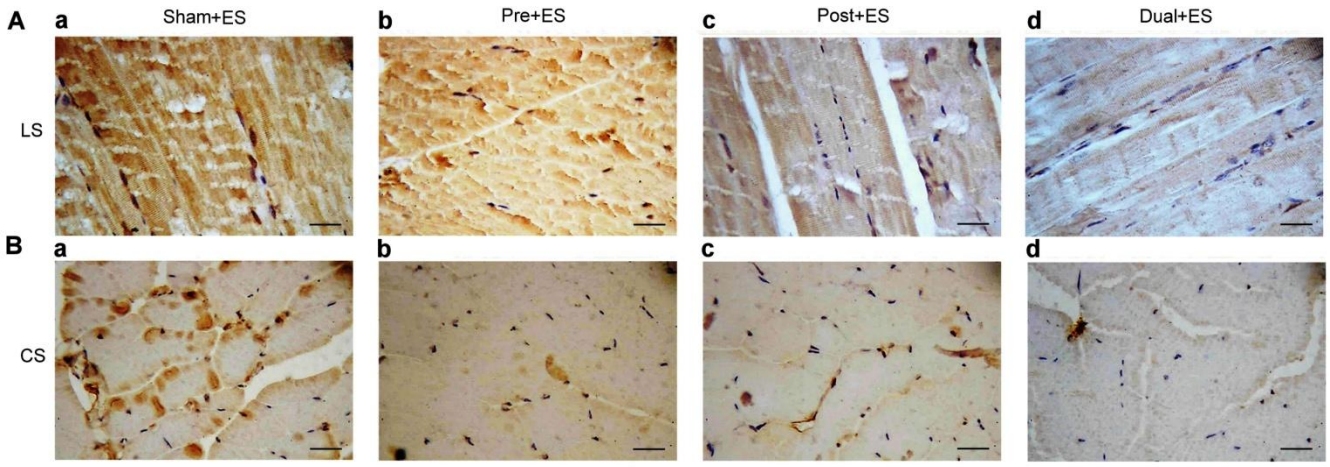


Fig. 8: Representative immunohistochemistry-based NFκB expression of gastrocnemius muscle tissue between mice with and without B307 treatment. (A) Longitudinal sections and (B) cross-sections of gastrocnemius muscle tissue through IHC staining reveal NFκB expression (expressed by deep brown color) in Pre+ES, Post+ES, and Dual+ES mice (b–d) was obviously weaker than in Sham+ES mice (a). Bar scale = 30 μm.

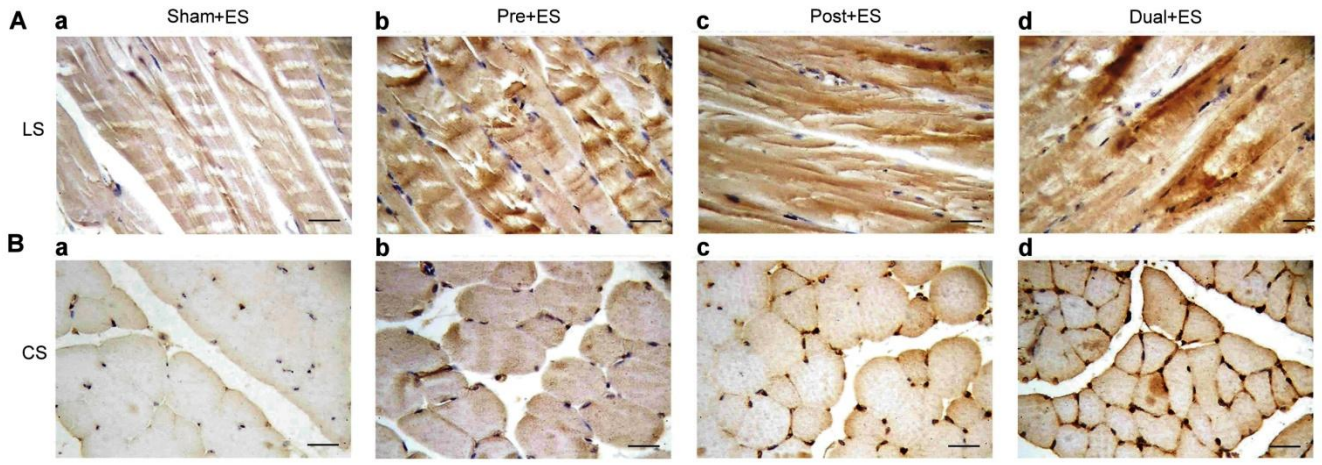


Fig. 9. Representative immunohistochemistry-based vinculin expression of gastrocnemius muscle tissue between mice with and without B307 treatment. (A) longitudinal sections and (B) cross-sections of gastrocnemius muscle tissue obtained through immunohistochemical staining. The vinculin expressions (expressed by a deep brown color) of Pre+ES, Post+ES, and Dual+ES mice (b–d) were markedly greater than those of Sham+ES mice (a). Scale bar = 30 μm.

Figures

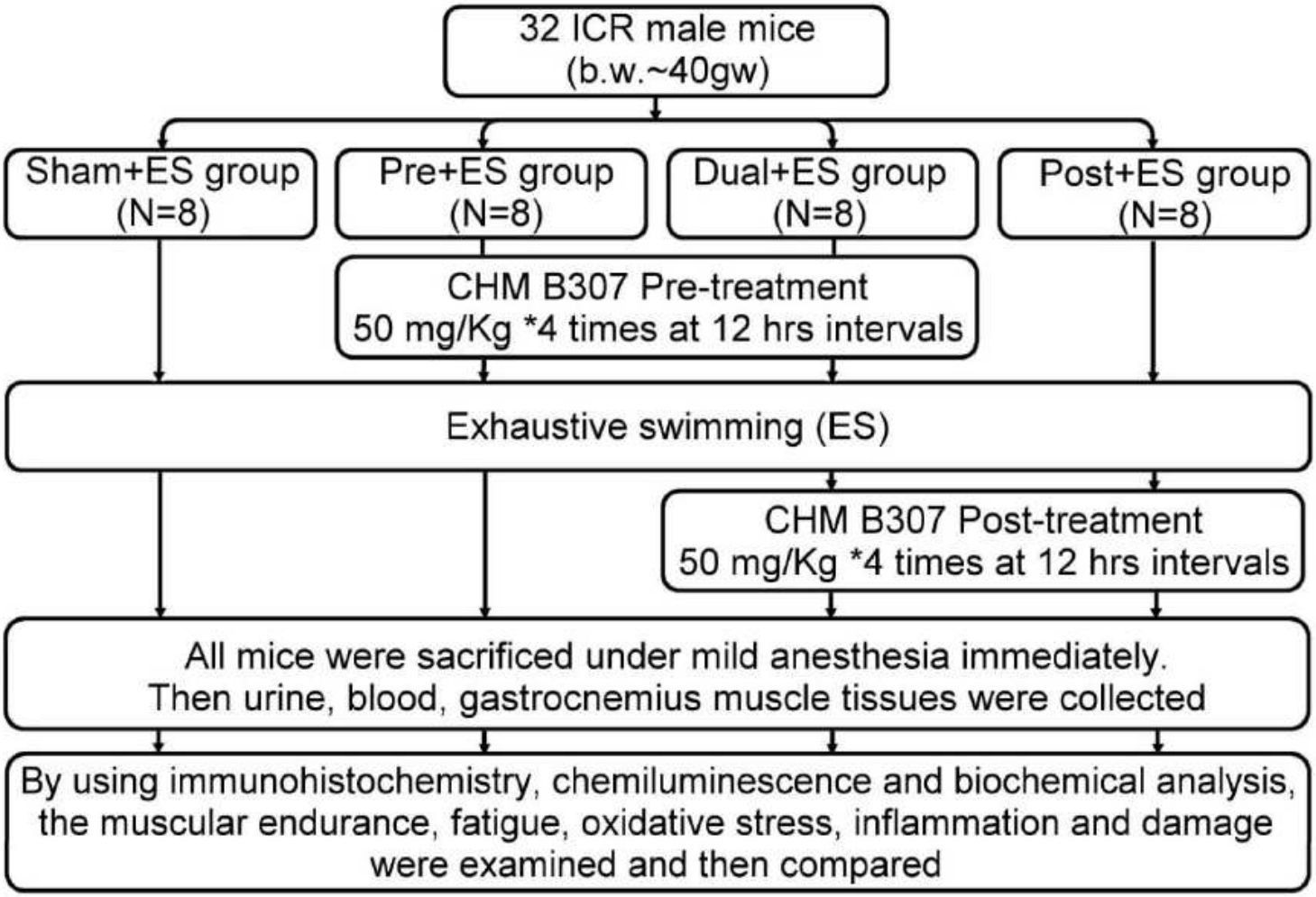


Figure 2

Study design for assessing the effects of supplemental Chinese herbal medicine B307 in ICR mice after exhaustive swimming (ES). Mice were subjected to ES under a forced swimming test. Thirty-two male ICR mice were randomly divided into 4 groups: Sham + ES, CHM B307 pretreatment + ES (Pre+ES), CHM B307 posttreatment + ES (Post+ES), and CHM B307 dual treatment + ES (Dual+ES).

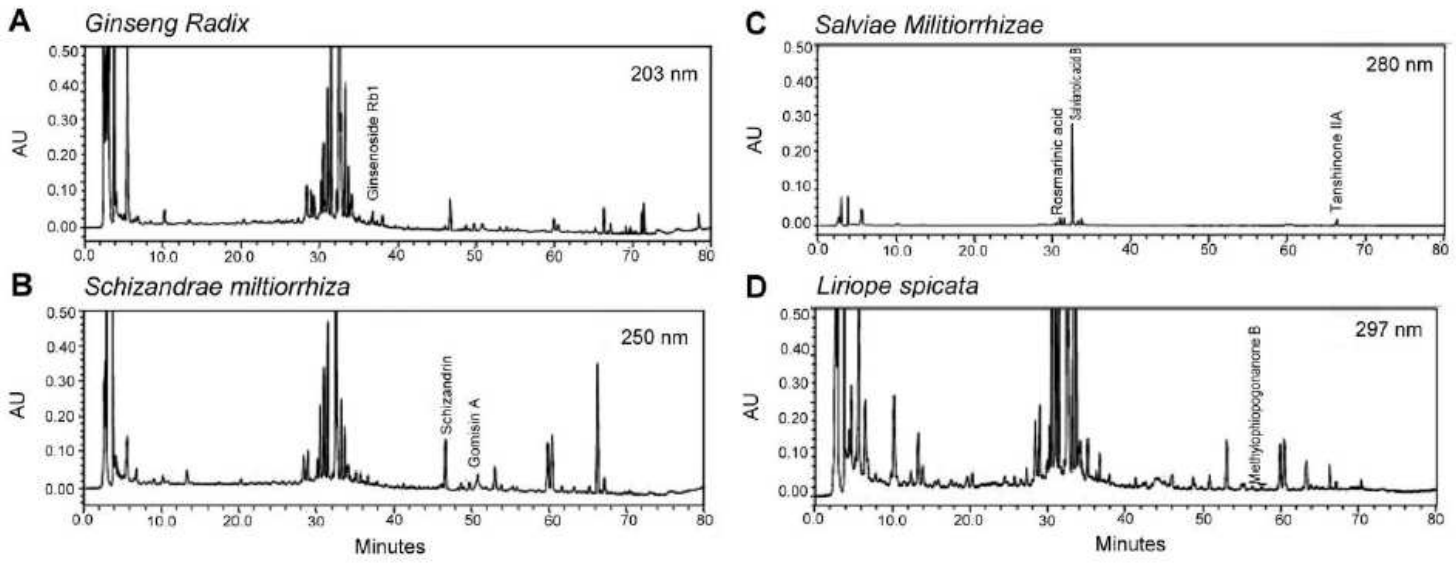


Figure 4

Chromatographic fingerprints of the Chinese herbal formula B307 from high-performance liquid chromatography. The peak for bioactive marker substances for (A) Ginseng Radix was ginsenoside Rb1(λ = 203 nm); (B) peaks for Schizandrae miltiorrhiza were schizandrin and gomisin A (λ = 250 nm); (C) peaks for Salviae Militiorrhizae Radix were rosmarinic acid, salvianolic acid B, and tanshinone IIA (λ = 280 nm); and (D) the peak for Liriope spicata was methylophiopogonanone B (λ = 297 nm).

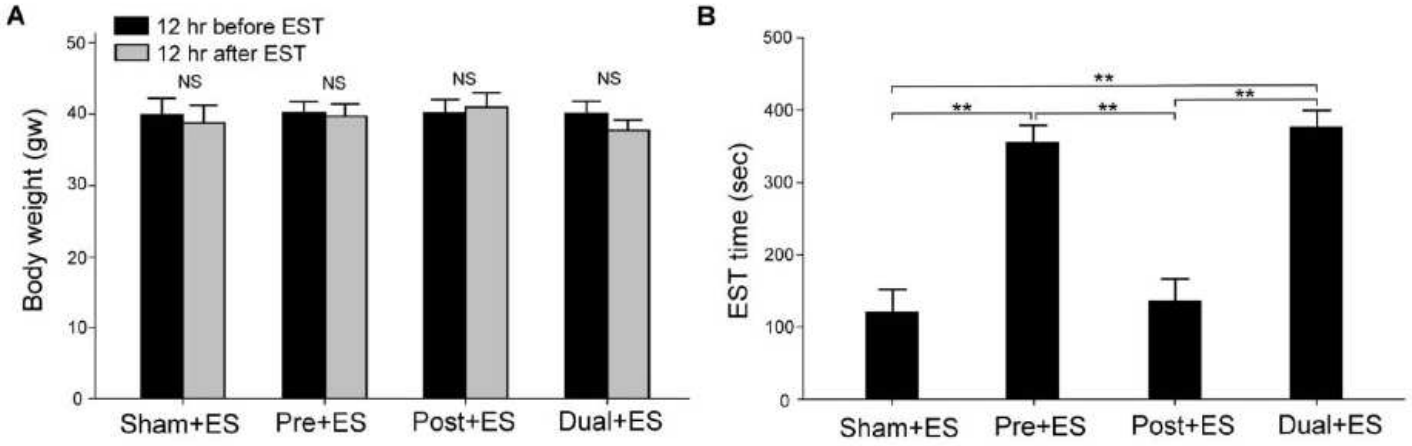
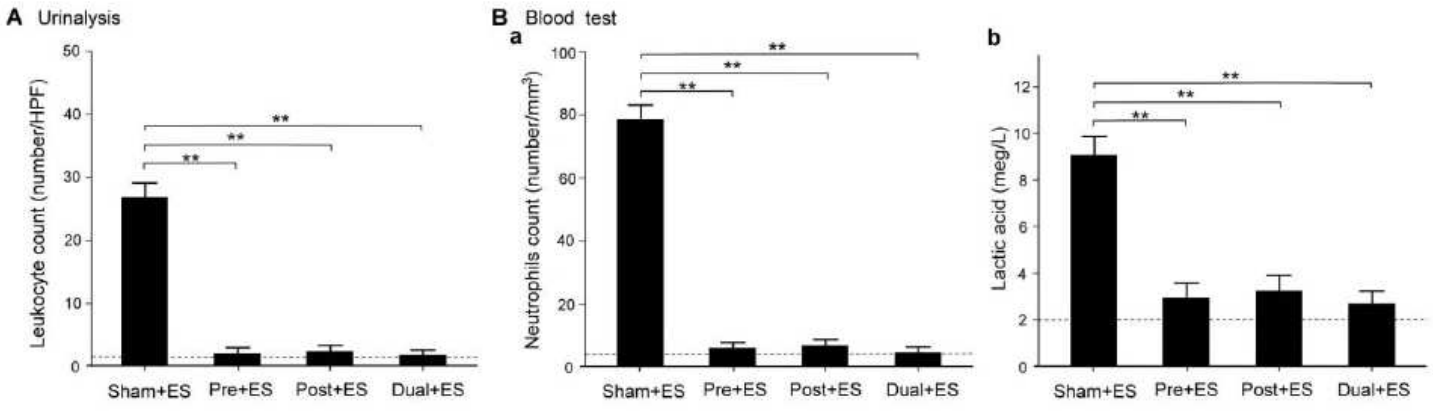


Figure 6

Comparisons of the body weight and ES time of mice with and without B307 treatment. (A) Statistical comparison of quantified body weight 12 h before and after the ES test. No differences (paired t test, $p > 0.05$) were noted among the Sham+ES, Pre+ES, Post+ES, and Dual+ES groups. (B) Statistical comparison of quantified ES time among groups. The data indicated that the ES times of Pre+ES and Dual+ES mice was significantly longer than those of Sham+ES and Post+ES mice (**$P < 0.01$, one-way ANOVA followed by Student–Newman–Keuls multiple comparison posttest). All data are shown as mean ± SEM.



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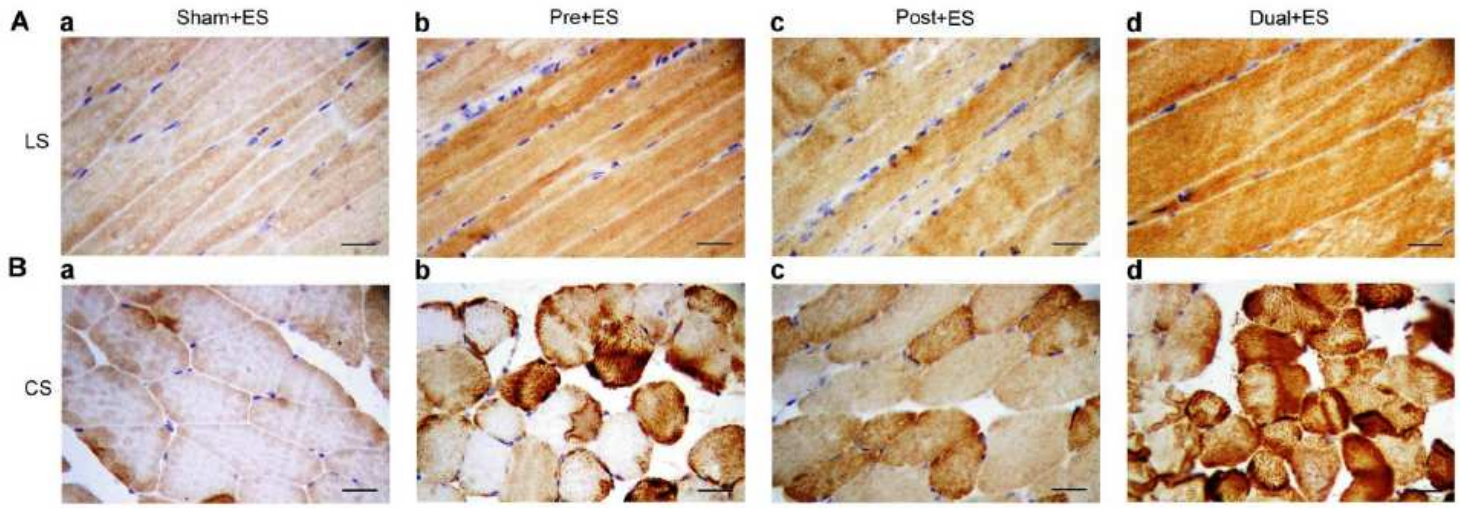
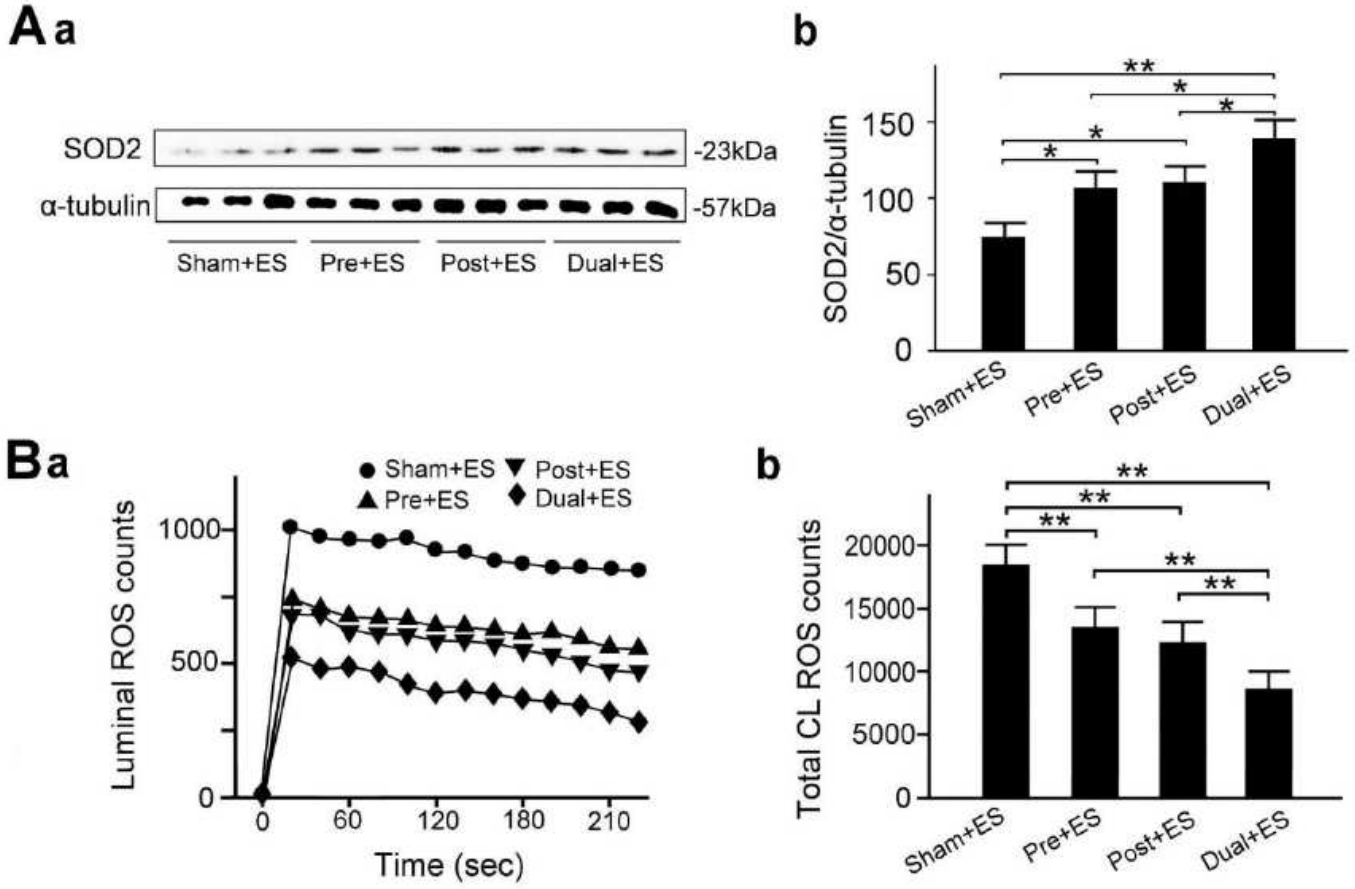


Figure 10

Representative immunohistochemistry-based SOD2 expressions of gastrocnemius muscle tissue between mice with and without Chinese herbal medicine B307 treatment. (A) Longitudinal sections and (B) cross-sections of gastrocnemius muscle tissue obtained through immunohistochemistry staining indicate that SOD2 expression (expressed by a deep brown color) in Pre+ES, Post+ES, and Dual+ES mice (b–d) was substantially higher than in Sham+ES mice (a). Scale bar = 30 μm.



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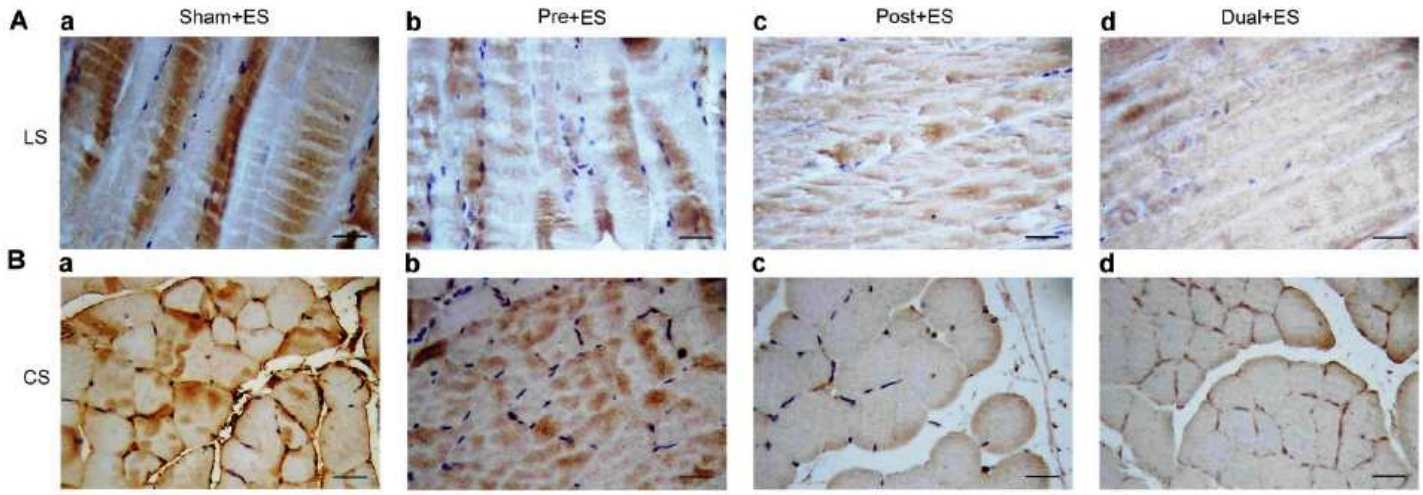
Comparisons of muscle SOD2 and blood reactive oxygen species (ROS) expression between mice with and without B307 treatment. (A) SOD2 expression of gastrocnemius muscle tissue among the Sham+ES, Pre+ES, Post+ES, and Dual+ES groups determined through Western blotting (a). Statistical comparison of quantified SOD2 expression among the groups (b). (B) Blood ROS expression among the groups determined through chemiluminescence analysis (a). Statistical comparison of quantified blood ROS expression among the groups (b). All data are shown as mean ± SEM (**P < 0.01, *P < 0.05, two-way ANOVA followed by Student–Newman–Keuls multiple comparison posttest). Experiments were repeated 3 times for each treatment.



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Representative immunohistochemistry-based TNF-α expression of gastrocnemius muscle tissue in response to ES between mice with and without B307 treatment. (A) Longitudinal sections and (B) cross-sections of gastrocnemius muscle tissue; TNF-α expression (expressed by a deep brown color) in Pre+ES, Post+ES, and Dual+ES mice (b–d) was markedly weaker than in Sham+ES mice. Scale bar = 30 μm.

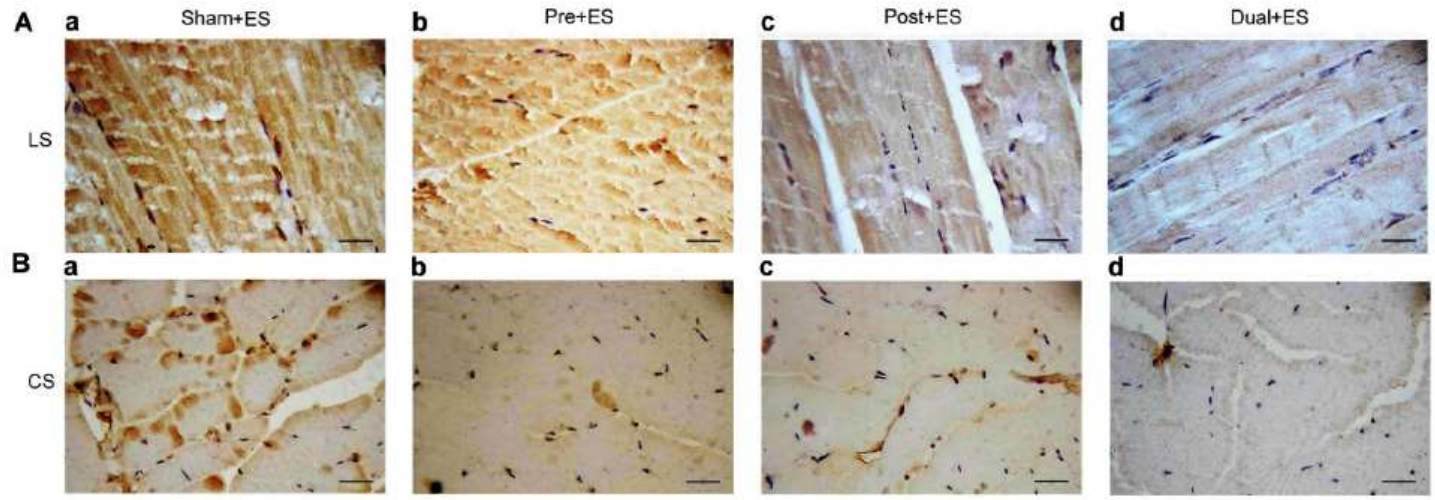

Figure 16

Representative immunohistochemistry-based NFκB expression of gastrocnemius muscle tissue between mice with and without B307 treatment. (A) Longitudinal sections and (B) cross-sections of gastrocnemius muscle tissue through IHC staining reveal NFκB expression (expressed by deep brown color) in Pre+ES, Post+ES, and Dual+ES mice (b–d) was obviously weaker than in Sham+ES mice (a). Bar scale = 30 μm.

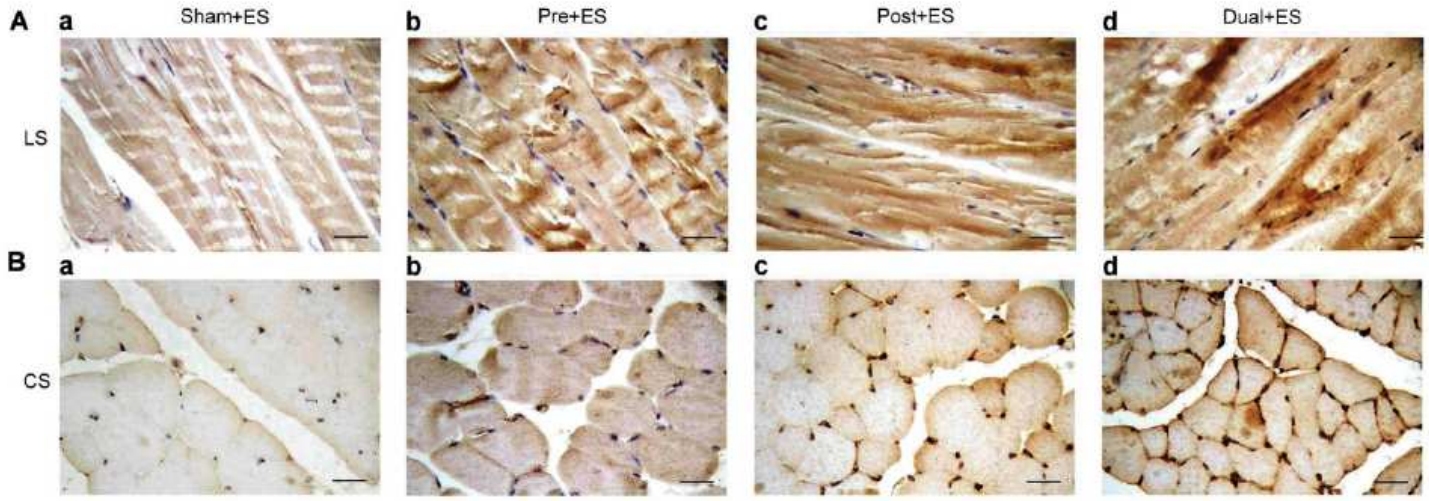

Figure 18

Representative immunohistochemistry-based vinculin expression of gastrocnemius muscle tissue between mice with and without B307 treatment. (A) longitudinal sections and (B) cross-sections of gastrocnemius muscle tissue obtained 560 through immunohistochemical staining. The vinculin expressions (expressed by a deep brown color) of Pre+ES, Post+ES, and Dual+ES mice (b–d) were markedly greater than those of Sham+ES mice (a). Scale bar = 30 μm.